ANNUAL RESEARCH PROGRESS REPORT

FY 1999

Grand Forks Human Nutrition Research Center

ANNUAL RESEARCH PROGRESS REPORT

(FY 1999)

GRAND FORKS HUMAN NUTRITION RESEARCH CENTER

UNITED STATES DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESEARCH SERVICE
NORTHERN PLAINS AREA

GRAND FORKS, NORTH DAKOTA 58202

MINERAL NUTRIENT REQUIREMENTS

MANAGEMENT UNIT

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149753 Year: 99 Project Number: 5450-51000-012-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Title: EFFECTS OF COPPER DEPLETION ON CARDIOVASCULAR FUNCTION AND METABOLISM

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Diets in the U.S. frequently are low in copper in comparison to dietary standards set by the Food and Nutrition Board of the National Academy of Sciences; these diets resemble the low copper diets that have produced abnormal electrocardiograms, increased cholesterol in blood, impaired metabolism of sugar, and poor control of blood pressure in men and women in controlled experiments. Diets low in copper may contribute to human illness (See Question 2).
The mission of this research program is to identify new biochemistry and physiology of copper with animal experiments to provide functional markers useful in dietary experiments with human volunteers and in community studies. The experiments will identify mechanisms by which adequate dietary copper produces beneficial effects, will identify new effects of human diets low in copper and will contribute to the establishment of national dietary standards.

2. How serious is the problem? Why does it matter?

The major signs of copper deficiency found in depleted men and women and deficient animals resemble the most common characteristics that can predict risk of ischemic heart disease in people. More than 75 anatomical, chemical and physiological similarities between animals deficient in copper and people with ischemic heart disease have been identified. It seems likely that the low copper diet common in the U.S. contributes to this disease which is the leading cause of death in the U.S., 480,000 deaths annually. The cost of medical care for this illness is more than $5 billion per year which does not include effects of sorrow, time lost from work or annual cost of prevention (at least $1000 per person). Proper selection of foods may yield diets that meet the standards mentioned above and prevent both illness and expense.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

Program 107 Human Nutrition Requirements, Food Composition and Intake equally divided between two components: a. Develop information about the effects of mild deficiency or imbalance of specific nutrients on

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biochemical, physiologic, and psychological functions to facilitate their detection and prevention and to define requirements for optimal health and performance for adults during the prime and latter years of life and b. Establish safe and optimal levels for dietary components and their roles in risk or prevention of diet-related disorders to provide comprehensive information about the effect of diet on chronic disease risk.

4. What were the most significant accomplishments this past year?

Methods of detecting low nutritional status for important trace elements are both imperfect and unreliable. Because hair analysis is being used to plan diets and to guide the use of nutritional supplements, multiple elements were measured in the hair of a man collected over two decades. Variability for both nutritional and toxic elements was so large even under these tightly controlled dietary and environmental conditions that if some of the high or low values had been obtained in isolation, intoxication or deficiency might have been inferred. These findings demonstrated that health status should not be inferred from hair analysis without supporting data from other sources.
Best chemical forms and doses of dietary supplements are ill defined. A study of acute effects of copper in drinking water was done. The threshold for copper sulfate is approximately 4 mg/l which exceeds the U.S. regulatory level of 1.3 mg/l. These data will be useful in providing data for dietary supplementation trials and for agencies that regulate water quality.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

A disparity between actual copper intakes and both copper requirements and dietary recommendations has been recognized from the publication by this author of dietary data pooled from 10 research groups in four countries to reveal 60% of daily diets contain less than 1.5 mg of copper and one third contain less than 1 mg. These results refute earlier nutritional beliefs that "adults generally consume 2 to 5 mg of copper daily."
High blood pressure from copper deficiency may be explained partially by the finding of impaired defense against damage by oxygen such as cholesterol oxidation and a related impaired ability to relax blood vessels.
Defense against oxidative damage that depends on adequate dietary copper is impaired in copper deficiency, where, however, genetic control of defense based on manganese is improved. This finding may identify a partially compensating mechanism.
Aortic aneurysms are a leading cause of death in men over age 50; low copper diets may contribute to aneurysm development (aneurysms are pathological enlargements of arteries that sometimes burst, an event that can be fatal).

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All of these findings are related to the mechanisms by which the Western diet gradually damages arteries and hearts leading to ischemic heart disease (see Question 2). Improving diets low in copper by selection of foods high in copper and by decreasing intake of foods low in copper is likely to have great benefits to both health and to decreasing the vast annual expenditures on medical care in middle and old age (see Question 2). Regular consumption of diets adequate in copper may lengthen life and assist in attaining a healthier old age. The ARS Food Pyramid is a useful guide.

6. What do you expect to accomplish, year by year, over the next 3 years?

During FY2000 the effects of creatine, a common dietary supplement, on copper utilization will be tested for cholesterol lowering ability and copper enhancing ability in animals because of its cholesterol lowering property in people. The ability of fluoride to modify the harmful effects of copper deficiency on bone strength will be tested similarly. We hope to publish our data on the use of electrocardiograms to define the magnesium requirement. Toward the end of FY 2000 and early in FY 2001 we will begin to use genetic typing to study nutritional requirements. Several candidate genes have been identified, the most promising of which controls a zinc enzyme related to both heart disease mortality and physical fitness. Zinc intakes are known to greatly affect copper utilization. A pilot study will be designed using genetic typing so that genetic effects on measurements of trace element status can be sought in appropriate target populations in FY 2002.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The United States Pharmacopeial Convention (USP) provides standards to the Food and Drug Administration in efficacy, quality and safety, etc., of prescription medicines. Dr. Klevay is a member of the USP Advisory Panel of Nutrition and Electrolytes which writes approximately a dozen nutritional monographs each year. These are incorporated into annually revised volumes published by the USP being immediately available to physicians and pharmacists and other regulatory agencies such as state medical boards.
A foundation and a marketing group enquired about the use of copper supplements in treatment of muscle and joint pain.
Preliminary data on the effects of copper in drinking water (See Question 4) were presented to the Environment Program Committee of the International Copper Association; these data may have immediate impact.
A lecture on "The development of the copper deficiency theory of ischemic heart disease" was given at the Medical College of Ohio.

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Information on the amounts of Vitamin C supplements likely to interfere with copper utilization was provided for inclusion in a forthcoming book on healthy diets.
Individual citizen inquiries on the benefits of plant rich diets, copper in drinking water and health effects of copper plumbing were answered.

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

An article "Lack of an RDA for copper may be hazardous to your health" published last year has been mentioned favorably in Self and the Dallas Morning News. Collaborative work on effects of plant rich diets on trace mineral status was noted in the Dallas Morning News, Longevity, and in the Newsletter of the Nutrition for Optimal Health Association. The overall project was favorably featured in the Grand Forks Herald.

9. Scientific Publications:

01. KLEVAY, L.M., CHRISTOPHERSON, D.M. and SHULER, T.R. 1999. Multiple toxic elements in hair of one man over two decades. FASEB J. 13: Abstr. p. A222.

02. KLEVAY, L.M., CHRISTOPHERSON, D.M. and SHULER, T.R. 1999. Variability of multiple nutritional elements in hair of one man over two decades. (TEMA-10) 10th Intl. Symp. on Trace Elements in Man and Animals, Abstr. p 449.

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400377 Year: 99 Project Number: 5450-51000-020-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: HOMEOSTASIS AND BIOAVAILABILITY OF TRACE ELEMENTS IN HUMANS AND ANIMALS

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

This CWU is oriented towards solving two major problems, one associated with manganese nutrition and the other associated with selenium nutrition.
A. Manganese is an essential nutrient and animals develop severe bone and leg abnormalities when insufficient dietary manganese is available. Despite many studies attempting to deplete manganese in humans, there has not been an unequivocal case of human manganese deficiency. Manganese is also a toxic element, but most reports of toxicity have been confined to employees in mines who breathe in large quantities of manganese-laden dust. Although unlikely, it has not been conclusively demonstrated that diets high in manganese do not induce toxicity or predispose humans to toxicity. Therefore the questions we are attempting to resolve are: Is there concern for either a deficiency or excess of manganese in humans consuming normal Western diets? What factors exacerbate or inhibit the uptake and/or retention of manganese from meals?
Approach: Human and animal studies are being and will be used to answer this question. Human studies use either free-living subjects or subjects living in a metabolic ward. Subjects are fed diets composed of normal foods but carefully formulated to deliver a particular amount of manganese. Because iron may interfere with manganese metabolism, some studies will utilize volunteers with different amounts of iron stores. Measures of enzymes and concentrations of nutrients in blood will be determined to assess the effect of dietary manganese on overall health. Radioisotopes will be used to determine the absorption and retention of manganese.
Animal studies will utilize laboratory animals or pigs fed diets with different amounts of manganese. Some animals will be surgically altered to allow access to body tissues or fluids that are normally inaccessible (e.g. bile).
B. Selenium is an essential element that has been demonstrated to have many health benefits to humans. The health benefits of selenium depend on the chemical form and the amount of selenium consumed; however, little work has been done with food forms of selenium. The question that is being addressed is: What is the best food form of selenium and what are its associated health benefits?
Approach: The health benefits of three food forms of selenium will be determined: selenium in broccoli grown to have high concentrations of selenium, selenium in meat and selenium in wheat. The primary variable that will be determined in laboratory animals is the prevention of colon

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National Program(s): 107 100%

cancer. Human studies will investigate the bioavailability, neuro-psychological benefits and selenium status of people consuming these forms of selenium.

2. How serious is the problem? Why does it matter?

Regulatory agencies have received conflicting information on the optimal intakes of manganese. Presently, the estimated safe and adequate daily dietary intake (ESADDI) overlaps the reference dose (the estimated amount of an element/compound that can be safely consumed on a daily basis over a lifetime) for manganese under some circumstances. Proper dietary guidance requires resolution of this problem.
Selenium supplementation has been found to reduce the incidence of many important cancers such as colon (third most common cause of cancer in men and women worldwide) and prostate (fourth most common cause of cancer in men worldwide) cancer. Inadequate selenium intakes are a serious problem in many parts of the world and some studies now suggest that residents of the USA may receive health benefits by consuming supplemental selenium. Presently there are limited options for supplementing dietary selenium intake.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This research relates to National Program 107, Human Nutrition Requirements, Food Composition and Intake; bioavailability of nutrients in food.

4. What were the most significant accomplishments this past year?

A. A study was conducted to determine whether iron stores affected the utilization of manganese by humans. Thirty human volunteers with low or high stores of iron were fed (at the Grand Forks Human Nutrition Research Center) diets that supplied high or low amounts of manganese. This study conclusively demonstrated that iron stores are a primary determinant of the amount of manganese absorbed from a meal, but retention of manganese was regulated independently of absorption by means of variable excretion. These results show the need to consider the iron stores of a human when attempting to determine the optimal intake of manganese.
Because fat type affects iron metabolism, and iron affects manganese metabolism, a study was conducted with rats that determined whether saturated, as compared to polyunsaturated, fats affect manganese absorption, distribution and retention. The rats were fed diets that contained either stearic acid (a saturated fat) or safflower oil (a polyunsaturated fat), administered a dose of radioactive manganese and total body radiation was measured for 10 days. Saturated fats depressed manganese absorption but did not have much effect on retention. These

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results indicate that fat type does influence how much manganese enters the body, but the amount of manganese that is ultimately retained is subject to further controls.

Because manganese and magnesium often have overlapping functions, and low dietary magnesium may result in heart abnormalities, we conducted a study to determine whether luxuriant amounts of manganese interfere with magnesium and heart function. Swine were fed diets deficient in magnesium and either adequate or high in manganese, and the retention and distribution of manganese and magnesium was determined. Many of the swine fed the low-magnesium/high-manganese died of heart failure and the amount of magnesium in the heart was reduced. This study shows that manganese can interfere with magnesium metabolism and perhaps contribute to the development of severe health problems.

Because broccoli may contain a unique and beneficial form of selenium but little is known about its metabolism, a study was conducted to determine the effectiveness of high-selenium broccoli in restoring tissue selenium concentrations and selenoprotein activities. Selenium deficient rats were re-fed selenium as a salt, selenomethionine and/or high-selenium broccoli. Selenium from broccoli accumulated slower and did not increase selenoprotein enzyme activity as well as other forms of selenium. These results indicate that the selenium from broccoli may not pose as much of a toxicological risk as other forms of selenium, and thus may be a good means of supplementing selenium.

Because rat studies indicate broccoli may contain a beneficial form of selenium, a study was conducted to determine how humans metabolize selenium from broccoli. Growing broccoli was loaded with a stable isotope of selenium and then fed to humans. Selenium from broccoli did not accumulate in the plasma as much as selenium from a salt, but less was excreted in the urine. These results indicate that selenium from broccoli may not pose much of a toxicological risk to humans because it does not accumulate in tissues to the extent that other forms of selenium do.

A study was conducted to determine which forms of selenium were most beneficial in suppressing indicators of colon cancer. Rats were fed diets that contained different forms of selenium, and were injected with a carcinogen. Salt forms of selenium, but not selenomethionine suppressed indicators of colon cancer. These data demonstrate that selenium suppresses colon cancer, but the suppressive effect depends on the form of the selenium.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

A. Based on the hypothesis that manganese absorption is primarily a by-product of iron absorption and that factors affecting iron absorption are the greatest determinant of iron absorption, a series of human studies has concluded that women with low iron status absorb more manganese than women with high iron status, but when greater amounts of manganese are absorbed,

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National Program(s): 107 100%

more manganese is excreted. Consequently, the human body seems to effectively regulate manganese retention under various physiological and dietary conditions.
Reported that selenium in broccoli is retained and distributed by rats and humans differently than other forms of selenium. Reported that precancerous lesions indicative of colon cancer are inhibited by selenium in a pattern dependent on chemical form and dose. Demonstrated that stable isotopes of selenium can be used to follow selenium incorporation into proteins. Used stable selenium to assess the effect of selenium supplementation to a selenium-deficient population.

6. What do you expect to accomplish, year by year, over the next 3 years?

Year 2000: In the year 2000 the study of high manganese intakes with different fat types in humans will be completed. Results of that study will be reported in a peer-reviewed journal. The preliminary evidence of high manganese intakes interfering with magnesium utilization and precipitating heart problems will be followed up with a study that examines the effects of similar diets on the microscopic anatomy of the heart.
Whether selenium in broccoli suppresses incidence of indicators of colon cancer will be determined. Whether different strains of broccoli accumulate different amounts of selenium will be determined.
Year 2001: Whether high manganese/low magnesium interferes with heart rate and rhythm will be determined. The studies of stearic acid inhibition of manganese absorption will be followed up with a mechanistic study designed to determine whether decreased absorption is associated with changes in expression of the iron-regulatory protein or NRAMP protein. A human supplementation study will be designed to assess the health benefits of the three food forms of selenium. Animal studies will be performed to ascertain the effectiveness of high-selenium wheat and meat in preventing colon cancer.
Year 2002: In vitro experiments will be conducted to determine how manganese may interfere with magnesium biochemistry in cardiac muscle. We will finish a human study designed to assess the health benefits of various food forms of selenium. A process for production of the most effective form of high selenium broccoli and meat will be developed.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The science of these studies has been transferred to the public by scientific publications.

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National Program(s): 107 100%

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

"Designer foods: a quick fix?" was published in the Grand Forks Herald.

9. Scientific Publications:

01. FENG, Y., FINLEY, J.W., DAVIS, C.D., BECKER, W.K., FRETLAND, A.J. and HEIN, D.W. 1999. Dietary selenium reduces...crypts in rats administered 3,2'-dimethyl-4-aminobiphenyl. Toxicol. Appl. Pharmacol. 157:36-42.

02. FINLEY, J.W. 1999. Manganese absorption and retention by young women is associated with serum ferritin concentration. Am. J. Clin. Nutr. 70:37-43.

03. FINLEY, J.W. 1999. The retention and distribution by healthy young men of stable isotopes of selenium consumed as selenite,...or hydroponically-grown broccoli are dependent on the isotopic form. J. Nutr. 129:865-871.

04. FINLEY, J.W. and DAVIS C.D. 1999. Protective effects of different chemical forms of Se on...crypt foci in the colons of rats with chemically induced carcinogenesis. Intl. Conf. on Diet and Prev. of Cancer, Abstr. p. P3.2.

05. FINLEY, J.W. 1999. The interaction between dietary fat type and dietary manganese concentration affects manganese absorption and retention in rats. FASEB J. 13: Abstr. p. A545.

06. MILLER, K.B. and FINLEY, J.W. 1999. Manganese concentration in tissues of pigs fed high and low amounts of manganese with and without trace mineral supplementation. FASEB J. 13: Abstr. p. A245.

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401877 Year: 99 Project Number: 5450-51000-020-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: SELENIUM IN NORTH DAKOTA BEEF

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Because of its reported health benefits, many people today are attempting to increase their dietary intake of the essential nutrient selenium. Beef is the single greatest source of dietary selenium for North Americans. North Dakota has soils high in selenium and may produce beef enriched in the nutrient. Thus, there is a need to determine whether beef grown in North Dakota is enhanced in selenium and whether there are health benefits associated with consuming selenium in this form.
Approach: The problem is being resolved by a series of field and laboratory studies that are determining the selenium content of ND beef, whether this selenium content can be enhanced by feeding high-selenium agricultural products (e.g. wheat, hay, etc.), and whether the beef produced in this manner has health benefits.
This project is also being supported by CRIS projects #5450-51000-020-02T and 5450-51000-020-03T.

2. How serious is the problem? Why does it matter?

The amount and chemical form of selenium in the diet is of great concern to nutritionists because selenium deficiency results in a fatal disease in China, and selenium supplementation has been demonstrated to drastically reduce the incidence of prostate (as well as lung and colo-rectal) cancer in the US. Prostate, lung and colo-rectal cancer are among the top five causes of cancer death worldwide.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This relates to National Program 107, Human Nutrition Requirements, Food Composition and Intake; bioavailability of nutrients in food.

4. What were the most significant accomplishments this past year?

A study was conducted to determine whether areas of North Dakota with high concentrations of selenium in the soil produce cattle with high concentrations of selenium in their meat. Soil, grass and beef samples were obtained from five diverse geographical regions of North Dakota and

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Accession: 0401877 Year: 99 Project Number: 5450-51000-020-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

all samples were analyzed for selenium. Results of the study showed that some areas of North Dakota produced beef that was 3-5-fold greater in selenium content than the national average, and the selenium content of the beef was significantly associated with soil and forage selenium concentrations. These results show that beef raised in an area of high soil selenium is a potentially good source of supplemental selenium.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

The above is the only project accomplished for this CRIS.

6. What do you expect to accomplish, year by year, over the next 3 years?

In the year 2000, it is expected to demonstrate that beef fed high-selenium feeds in a feedlot will concentrate the selenium in muscle tissue. The health benefits of selenium partially depend on the chemical form in which it is consumed. Consequently, it cannot be assumed that beef enriched in selenium has enhanced health benefits. Therefore, in 2001 and 2002, the effectiveness of high-selenium beef in suppressing the incidence of colon cancer will be tested.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The science of these studies has been transferred to the public by: 1. Meetings and talks with ranchers and beef producers. 2. A talk at MarketPlace 99 - an agricultural expo held in Bismarck, ND ("High selenium beef: Does it enhance the marketability and/or profitability of beef?")

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

None.

9. Scientific Publications:

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0402745 Year: 99 Project Number: 5450-51000-020-02 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: PRODUCTION OF HIGH SELENIUM BEEF

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Because of its reported health benefits, many people today are attempting to increase their dietary intake of the essential nutrient selenium. Beef is the single greatest source of dietary selenium for North Americans. North Dakota has soils high in selenium and may produce beef enriched in the nutrient. Thus, there is a need to determine whether beef grown in North Dakota is enhanced in selenium and whether there are health benefits associated with consuming selenium in this form.
Approach: The problem is being resolved by a series of field and laboratory studies that are determining the selenium content of ND beef, whether this selenium content can be enhanced by feeding high-selenium agricultural products (e.g. wheat, hay, etc.), and whether the beef produced in this manner has health benefits.
This project is also being supported by CRIS projects #5450-51000-020-01S and 5450-51000-020-03T.

2. How serious is the problem? Why does it matter?

The amount and chemical form of selenium in the diet is of great concern to nutritionists because selenium deficiency results in a fatal disease in China, and selenium supplementation has been demonstrated to drastically reduce the incidence of prostate (as well as lung and colo-rectal) cancer in the US. Prostate, lung and colo-rectal cancer are among the top five causes of cancer death worldwide.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This relates to National Program 107, Human Nutrition Requirements, Food Composition and Intake; bioavailability of nutrients in food.

4. What were the most significant accomplishments this past year?

A study was conducted to determine whether areas of North Dakota with high concentrations of selenium in the soil produce cattle with high concentrations of selenium in their meat. Soil, grass and beef samples were obtained from five diverse geographical regions of North Dakota and

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Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

all samples were analyzed for selenium. Results of the study showed that some areas of North Dakota produced beef that was 3-5-fold greater in selenium content than the national average, and the selenium content of the beef was significantly associated with soil and forage selenium concentrations. These results show that beef raised in an area of high soil selenium is a potentially good source of supplemental selenium.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

The above is the only project accomplished for this CRIS.

6. What do you expect to accomplish, year by year, over the next 3 years?

In the year 2000, it is expected to demonstrate that beef fed high-selenium feeds in a feedlot will concentrate the selenium in muscle tissue. The health benefits of selenium partially depend on the chemical form in which it is consumed. Consequently, it cannot be assumed that beef enriched in selenium has enhanced health benefits. Therefore, in 2001 and 2002, the effectiveness of high-selenium beef in suppressing the incidence of colon cancer will be tested.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The science of these studies has been transferred to the public by: 1. Meetings and talks with ranchers and beef producers. 2. A talk at MarketPlace 99 - an agricultural expo held in Bismarck, ND ("High selenium beef: Does it enhance the marketability and/or profitability of beef?")

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

None.

9. Scientific Publications:

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0402749 Year: 99 Project Number: 5450-51000-020-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: HIGH SELENIUM BEEF PRODUCED IN NORTH DAKOTA

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Because of its reported health benefits, many people today are attempting to increase their dietary intake of the essential nutrient selenium. Beef is the single greatest source of dietary selenium for North Americans. North Dakota has soils high in selenium and may produce beef enriched in the nutrient. Thus, there is a need to determine whether beef grown in North Dakota is enhanced in selenium and whether there are health benefits associated with consuming selenium in this form.
Approach: The problem is being resolved by a series of field and laboratory studies that are determining the selenium content of ND beef, whether this selenium content can be enhanced by feeding high-selenium agricultural products (e.g. wheat, hay, etc.), and whether the beef produced in this manner has health benefits.
This project is also being supported by CRIS projects #5450-51000-020-01S and 5450-51000-020-02T.

2. How serious is the problem? Why does it matter?

The amount and chemical form of selenium in the diet is of great concern to nutritionists because selenium deficiency results in a fatal disease in China, and selenium supplementation has been demonstrated to drastically reduce the incidence of prostate (as well as lung and colo-rectal) cancer in the US. Prostate, lung and colo-rectal cancer are among the top five causes of cancer death worldwide.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

This relates to National Program 107, Human Nutrition Requirements, Food Composition and Intake; bioavailability of nutrients in food.

4. What were the most significant accomplishments this past year?

A study was conducted to determine whether areas of North Dakota with high concentrations of selenium in the soil produce cattle with high concentrations of selenium in their meat. Soil, grass and beef samples were obtained from five diverse geographical regions of North Dakota and

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Report of Progress (AD-421)

Accession: 0402749 Year: 99 Project Number: 5450-51000-020-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

all samples were analyzed for selenium. Results of the study showed that some areas of North Dakota produced beef that was 3-5-fold greater in selenium content than the national average, and the selenium content of the beef was significantly associated with soil and forage selenium concentrations. These results show that beef raised in an area of high soil selenium is a potentially good source of supplemental selenium.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

The above is the only project accomplished for this CRIS.

6. What do you expect to accomplish, year by year, over the next 3 years?

In the year 2000, it is expected to demonstrate that beef fed high-selenium feeds in a feedlot will concentrate the selenium in muscle tissue. The health benefits of selenium partially depend on the chemical form in which it is consumed. Consequently, it cannot be assumed that beef enriched in selenium has enhanced health benefits. Therefore, in 2001 and 2002, the effectiveness of high-selenium beef in suppressing the incidence of colon cancer will be tested.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The science of these studies has been transferred to the public by: 1. Meetings and talks with ranchers and beef producers. 2. A talk at MarketPlace 99 - an agricultural expo held in Bismarck, ND ("High selenium beef: Does it enhance the marketability and/or profitability of beef?")

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

"Selenium could bring additional dollars to area farmers" was published in Farm and Ranch Guide, Feb. 26, 1999, pg. 32.

9. Scientific Publications:

01. HINTZE, K.J., FINLEY, J.W., LARDY, G.P., MARCHELLO, M. and SEDIVIC, K. 1999. Selenium content of beef produced in North Dakota varies by geographic region. J. An. Sci. 77(S1): Abstr. p. 206.

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400398 Year: 99 Project Number: 5450-51000-021-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: BIOAVAILABILITY OF TRACE ELEMENTS, ESPECIALLY IRON FROM FOOD, & ITS INFLUENCE ON NUTRITURE & FUNCTION

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Iron bioavailability, depending on the form of iron, and the presence of enhancers or inhibitors in foods, can vary 5-10 fold from meals with similar iron content, but does not substantially affect iron stores after such diets are fed for several weeks. Concerns about the adverse effects of iron deficiency on cognition and physical performance as well as concerns about high iron stores and the risk of chronic diseases (such as cancer and heart disease) emphasize the need to determine how dietary iron bioavailability should alter dietary advice for the public.
Both short-term iron absorption and longer-term iron status are being measured in humans consuming controlled diets for several weeks to help determine the true importance of dietary iron bioavailability, and the related impact on practical dietary choices such as consuming less meat, more beans and whole grains, or more tea. The bioavailability of other mineral nutrients, such as calcium, copper, and zinc, may also be affected by such dietary choices, and information on bioavailability of these other mineral nutrients can often be efficiently derived from the same human studies. The iron research will determine the practical importance of dietary iron bioavailability, and how extensively biological adaptation modifies it.

2. How serious is the problem? Why does it matter?

Iron nutrition is a double-edged sword. While adequate iron nutriture in infants, children, and childbearing age women is essential for cognitive development and work capacity, excessive body iron in men and postmenopausal women is hypothesized to increase oxidative stress and the risk of chronic diseases such as heart disease and cancer. Research on dietary iron bioavailability provides information for policy decisions about recommended dietary allowances, food enrichment and fortification standards, and dietary guidelines for the public.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 107, Human Nutrition Requirements, Food Composition, and Intake (100%).

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Accession: 0400398 Year: 99 Project Number: 5450-51000-021-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

This research is directly related to one of ten current nutrition problems and related objectives designated in the ARS National Programs. Specifically, this research concerns the problem, "the bioavailability of nutrients in food".

4. What were the most significant accomplishments this past year?

Iron intakes in the US have increased because of increased fortification of foods and use of nutrient supplements. To help understand how well the body adapts to changes in iron intake, we investigated how well men and women adapted to supplementation with 50 mg iron daily for 12 weeks. An increase in iron stores with supplementation persisted for 6 months only in men and women with iron stores that were already adequate to high, but not in those with low iron stores. These results indicate that people with adequate iron stores do not fully adapt to prevent increased iron stores with supplementation, but that women with low iron stores may need continuing iron supplementation to counterbalance high rates of iron excretion.
Intestinal ferritin production, as measured in fecal samples, may indicate how the body adapts to adjust iron absorption. Fecal ferritin excretion was measured in iron supplemented and control subjects. The results provide new information on the variability of ferritin excretion in a free-living population and indicate that the intestine rapidly increases or decreases ferritin production in response to increased or decreased dietary iron, consistent with adaptation that was observed in nonheme, but not heme iron absorption. Fecal ferritin observations will help us understand the intestinal control of iron absorption, and differences in the control of heme and nonheme iron absorption.
Hemochromatosis, a genetic human disease of excessive liver iron accumulation, can be modeled by mice with a mutation in beta-2 microglobulin and studying these mice can help us understand the body's control of iron stores. Dietary iron absorption and retention were measured in mutant and control mice. Mutant mice accumulated more iron in the liver without greater intestinal iron uptake or greater total body iron retention. These results indicate that high liver iron in hemochromatosis may be at least partially because of an abnormality in the tissue distribution of iron, rather than just an abnormality of iron absorption or excretion.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Determined that daily iron supplementation reduces the efficiency of nonheme, but not heme iron absorption from food by about 36%. Demonstrated that US men partially adapt to differences in dietary iron bioavailability, decreasing their iron retention to less than 1 mg iron per day, despite the high iron bioavailability of their customary diet. Found that adaptation in

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Accession: 0400398 Year: 99 Project Number: 5450-51000-021-00 D
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National Program(s): 107 100%

iron absorption occurred without a change in serum ferritin, the best noninvasive indicator of body iron stores in humans. Demonstrated that research with short-term diets overestimates differences in iron bioavailability between chronic diets. These new findings about adaptation in iron absorption can be helpful in setting recommended dietary allowances for iron.
Determined that women of child-bearing age absorbed substantially less (about 70% less) nonheme iron from a lacto-ovo-vegetarian, compared with an omnivorous diet. Documented that common measures of iron status were unaffected by consuming such a diet for eight weeks, but that fecal ferritin excretion was sensitively affected by dietary iron bioavailability. These findings emphasize the limited response of serum ferritin (a proposed risk factor for cardiovascular diseases and cancer) to dietary iron bioavailability, as well as providing a basis for dietary advice to vegetarians.
Demonstrated that copper, iron and zinc interact to influence body mineral status, that high dietary iron does not induce overt oxidative stress, and that indices of antioxidant capacity are primarily influenced by dietary copper in rats. The results suggest diets high in iron, within a range that could be expected for human diets with iron enrichment, fortification or supplementation, have minimal or no effect on biological oxidation risk factors for chronic disease.

6. What do you expect to accomplish, year by year, over the next 3 years?

Year 1: We will determine if previous observations of reduced serum copper and ceruloplasmin associated with a lactoovovegetarian diet can be explained by reduced copper absorption from copper-abundant vegetarian diets. We will determine the effect of body iron stores and of dietary calcium on mucosal uptake and serosal transfer of both heme and nonheme iron in humans. We will compare radiotracer and slope ratio bioavailability methods as part of a cooperative study of the bioavailability of zinc from refined and unrefined Philippine rice.
Year 2: We will determine if women of childbearing age adapt to dietary iron bioavailability, and whether adaptation is limited to the specific dietary enhancers and inhibitors in the chronic diet, or constitutes a more general change in absorptive efficiency. Detailed dietary assessment data on 300 women will determine whether dietary variables (such as meat) that affect short-term dietary bioavailability can be related to long-term iron stores. We will test the hypothesis that, unlike purified sources of protein, meat consumption does not reduce human body calcium retention.
Year 3: We will test a new experimental approach to determining zinc requirements in humans, by determining how people adapt their zinc absorption to different dietary zinc intakes. In the next 3-5 years, we will quantify iron excretion rates for women, based on isotopic dilution over a 3-year period, and will relate iron excretion to body iron stores.

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National Program(s): 107 100%

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

JR Hunt was invited to present "Zinc Intake, Absorption and Human Requirements" at the National Academy of Science, Food and Nutrition Board Forum on "Dietary Reference Intakes for Iron and Zinc", Washington, D.C., April 20, 1999.
Additional data on "Zinc Absorption From An Experimental Diet Based On The FDA Total Diet Study" (published in Nutrition Research 12:1335, 1992) was provided, at their request, to the National Academy of Science, Food and Nutrition Board subcommittee working on establishing dietary recommendations for zinc.
Research results concerning men's ability to adapt to dietary iron bioavailability (American Journal of Clinical Nutrition, in press) was provided, at their request, to the National Academy of Science, Food and Nutrition Board subcommittee working on establishing dietary recommendations for iron.
The new finding that people partially adapt to iron supplementation by reducing their iron absorption from food was transferred to several customer groups, including a preliminary report to the producer organization that partially sponsored the research, and presentations at the national Experimental Biology meeting in Washington, DC, the International BioIron meeting in Sorrento, Italy, and meetings of the North Dakota Academy of Science and the North Dakota Dietetic Association in Grand Forks, ND.

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

The American Journal of Clinical Nutrition highlighted our research on iron nutrition and vegetarian diets by publishing a favorable editorial by ER Monsen, May, 1999.
Our research on iron and depression was featured in an article by Judy McBride in the May issue of Agricultural Research.
JR Hunt presented "Vegetarian Diets: Can You Leave out the Meat, Eat More Beans and Grains, and Still Absorb Enough Iron, Zinc and Other Minerals?" at the Meeting of the North Dakota Dietetic Association, Grand Forks, ND, April 22, 1999.
ZK Roughead presented "Phytochemicals: The New Kids on the Block?" at the Pharmacy Homecoming Seminars, October 2, 1998, Fargo, ND
ZK Roughead presented "Herbal Supplements... Heal or Harm?" to the Greater Grand Forks Dietetic Association, March 4, 1999, Grand Forks, ND.

9. Scientific Publications:

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National Program(s): 107 100%

Publications: (Continued)

01. HUNT, J.R. and ROUGHEAD, Z.K. 1999. Nonheme iron absorption, fecal ferritin excretion, and blood indices of iron status in women consuming controlled lactoovovegetarian diets for eight weeks. Am. J. Clin. Nutr. 69:944-952.

02. HUNT, J.R. and PENLAND, J.G. 1999. Iron status and depression in premenopausal women: an MMPI study. Behav. Med. 25:62-68.

03. ROUGHEAD, Z.K. and HUNT, J.R. 1999. Iron supplementation reduces iron absorption from food. Proc. ND. Acad. Sci. 53:209.

04. HUNT, J.R. 1999. Vitamins and minerals: To supplement or not to supplement? Proc. ND. Acad. Sci. 53:73.

05. ROUGHEAD, Z.K., JOHNSON, L.K. and HUNT, J.R. 1999. Dietary copper primarily affects antioxidant capacity and dietary iron mainly affects iron status in ...varying concentrations of iron, zinc and copper. J. Nutr. 7:1368-1376.

06. ROUGHEAD, Z.K. and HUNT, J.R. 1999. Adaptation in iron absorption: Daily iron supplements reduce nonheme, but not heme iron absorption from a meat-based meal. FASEB J. 13: Abstr. p. A242.

07. ROUGHEAD, Z.K. and HUNT, J.R. 1999. Daily iron supplementation decreases absorption efficiency of nonheme, but not heme iron, form food and slightly increases serum ferritin in normal volunteers. BioIron '99 Mtg, Abst. #182.

08. HUNT, J.R. and ROUGHEAD, Z.K. 1999. Incorporation of absorbed iron into erythrocytes is inversely related to iron stores and is reduced by daily iron supplementation. BioIron '99 Mtg, Abst. #369.

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

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09/10/99

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401621 Year: 99 Project Number: 5450-51000-021-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: WHOLE BODY COUNTING AND RADIOTRACER METHODS IN RESEARCH ON MINERAL REQUIREMENTS IN HUMAN NUTRITION

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

To make dietary recommendations and evaluate dietary practices that promote good mineral nutrition for the population, there must be sensitive methods for measuring mineral nutrient absorption, excretion, retention, and food bioavailability. The use of isotopic tracer methodology can effectively contribute to meeting these needs. Specifically, use of a whole body scintillation counter can safely and sensitively determine retention of mineral elements that have gamma-emitting isotopes with short to moderate half-lives, such as cadmium, calcium, copper, iron, magnesium, manganese, and zinc. The whole body counting approach has the advantage of determining mineral retention without volunteer inconvenience, high variability, and incomplete sample collections associated with collecting mineral excretion data. It allows the use of a true tracer that does not alter the absolute mass of the mineral under investigation, and is easily and sensitively measured with minimal labor. This agreement provides the expertise of a certified health physicist to cooperate with nutrition scientists at the Grand Forks Human Nutrition Research Center, providing an interdisciplinary approach to answering nutrition questions with whole body counting methodology.

2. How serious is the problem? Why does it matter?

Nutrient bioavailability addresses the ability to effectively utilize the nutrients in food for biological functions. Two diets that contain similar amounts of a nutrient, such as iron, can differ by as much as 10-fold in the amount of iron nutrient that is absorbed, retained, and utilized. Internationally, zinc deficiency has been observed in humans whose diets contained considerable quantities of zinc, but that zinc was not bioavailable because of phytic acid from whole grains or legumes that interfered with absorption and lack of protein that enhances absorption. Domestically, there is concern that dietary trends (and some recommendations) to increase whole grains and legumes while reducing animal products in the diet may compromise copper, iron and zinc nutrition. The promotion of mineral supplements in the US may lead to imbalances that affect the absorption and retention of other minerals. To complement new knowledge of the importance of nutrients for optimal health, we must also know the bioavailability of and interactions among nutrients from common

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National Program(s): 107 100%

diets, in order to provide dietary advice to the public.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 107, Human Nutrition Requirements, Food Composition, and Intake (100%).
This research is directly related to one of ten current nutrition problems and related objectives designated in the ARS National Programs. Specifically, this research concerns the problem, the bioavailability of nutrients in food.

4. What were the most significant accomplishments this past year?

Radiotracer studies can allow the safe and sensitive measurement of mineral nutrient absorption and retention in humans. Whole body counting measurements were utilized to investigate a) adaptation in iron absorption in response to iron supplementation, and b) manganese absorption and retention, as influenced by dietary manganese and the saturation of dietary fat. Demonstrated that people supplemented with iron adapt to reduce the amount of iron they absorb from food, and that people consuming low manganese diets adapt to absorb manganese more efficiently. These findings can be useful in recommended dietary allowances. See CRIS 5450-51000-020-00D and 5450-51000-021-00D.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

The continuous improvement in whole body counting methods provides a unique resource for extending knowledge of mineral absorption and retention. As part of this monitoring and improvement, the greater natural gamma ray background radiation at the GFHNRC was quantitatively compared with that at Lawrence Livermore National Laboratory (LLNL), Livermore, California, by recording measurements using two Nal(TI) detectors both at GFHNRC and at LLNL. Continued monitoring and improvements to reduce background radiation will assure the sustained sensitivity of this unique equipment to support nutrition research.
Substantial work on the repair and replacement of equipment damaged by the 1997 Grand Forks flood has provided the opportunity for evaluation and improvement of methods and equipment. This included production of a new Uniform Isotope Source (UNIS) board, as well as preliminary planning to integrate a new algorithm employing a "three-gamma ray" UNIS board into Library Least Squares programs to improve data collection independent of subject size and radioisotope distribution.
Work has begun to upgrade older DOS-based computer programs to Windows-based user friendly software for use with the repaired small animal whole body counter.

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Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

An in-depth quality control study of the GFHNRC whole body counting equipment showed decreased sensitivity and increased resolution of whole body counter detectors over several years time. This evaluation resulted in a long-term plan for gradual refurbishment of detectors in the whole body counter, without interfering with ongoing nutrition studies. Recent equipment improvements enhance the support of research at the GFHNRC. Gamma radiation detection equipment with highly sensitive germanium detectors, obtained on loan from the North Dakota State Department of Health and Consolidated Laboratories, will improve analytical standardization of isotope sources to be used in nutrition studies. A large 12.5 cm x 5 cm NaI(TI) cylindrical detector was obtained, allowing selective measurement of cranium and other organ gamma ray emissions in radioactive trace studies.
Precise whole body counting measurements allowed sensitive measurements of iron retention, revealing that men adapt iron absorption in response to changes in dietary iron bioavailability. These results demonstrated that research with short-term diets overestimates differences in iron bioavailability between chronic diets. The information can be helpful in setting recommended dietary allowances for iron.

6. What do you expect to accomplish, year by year, over the next 3 years?

The library least squares methodology will be further developed to establish minimal detection limits for specific radioisotopes measured against a typical K-40 background, and to utilize the new algorithm based on the "three-gamma ray" UNIS board. This work will aid data analysis and interpretation of the variation caused by body size and density in self-absorption of internally produced radiation. Improved precision of whole body counting will result, especially for persons with varying body size and shape, and whole body counting will be more useful in determining changes in body composition associated with diet and/or exercise over time. In addition, the newly acquired HpGe detector will be utilized in the standardization and use of Ca-47 studies of the effect of dietary protein on calcium retention.
Year 2: We will develop procedures to use the HpGe detector to measure low activity biological samples (such as blood iron-59 and zinc-65 after labeled meals), allowing us to assess and model the distribution of trace elements after absorption. New standardization procedures will be developed and implemented for the small animal counter to improve accuracy by utilizing multi-gamma standards and "commercial" calibration programs. We will also adapt the "commercial" calibration programs to the meal counter and apply the HpGe detector to monitor the accuracy of the meal counter measurements.
Year 3: We will further improve total body potassium (TBK) measurements for various body shapes and in the presence of internal interfering gamma ray emitting isotopes.

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Accession: 0401621 Year: 99 Project Number: 5450-51000-021-01 S
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Because of the uniqueness of the whole body counting equipment and technology, the major transfer of technologies from the whole body counter work have occurred through reports and publications of the results of the major nutrition projects which employed the counter, and are reported under those specific CRIS reports. In addition, other research centers have adopted this original counter design.
A perquisite spin-off from studies of the steel room background radiation has led to research into contamination of lead used in contacts on high speed, high density semiconductor computer memory elements. Radiation from contaminated lead contacts leads to errors in the memory elements ("soft errors"). This work is supported by The Ballistic Missile Defense Organization through the Department of Defense Experimental Program to Stimulate Competitive Research (DEPSCoR). Clean lead contacts, "solder bumps" in 'flip chip' memory elements will result in faster, smaller, more accurate computer memories.

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

Along with his Physics REU student, Dr. Lykken presented a poster entitled A study of factors affecting whole body counter steel room background at the North Dakota Science, Engineering & Mathematics 7th Annual POSTER SESSION PROGRAM, NDSU, Fargo, ND, July 28, 1999.

9. Scientific Publications:
01. MOMCILOVIC, B. and LYKKEN, G.I. 1998. 54Mn gastrointestinal absorption and tissue distribution in brain and liver. KNJIGA SAZETAKA RADOVA, p 81, 82.

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0401160 Year: 99 Project Number: 5450-51000-021-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

Title: ADAPTATION IN THE ABSORPTION OF IRON FROM BEEF

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Concerns about high iron stores and the risk of chronic diseases such as heart disease and cancer have led to speculation that red meat intake should be limited because it is an excellent source of highly absorbable iron. There is special concern that heme iron absorption is not biologically controlled to the same degree as nonheme iron absorption. This project is related to parent CRIS 5450-51000-021-00D by the same investigators, and is limited to a single human experiment that has been partially funded by the National Cattlemen's Beef Association. This experiment will determine a) whether heme iron absorption from a meat-based meal is reduced after iron supplementation, b) whether nonheme iron absorption from a meat-based meal is reduced after iron supplementation, c) whether intestinal ferritin production, as measured in fecal samples, is increased after iron supplementation, and is associated with changes in heme and nonheme iron absorption, and d) whether serum ferritin is increased substantially after iron supplementation, and if any increase in serum ferritin persists after iron supplementation is discontinued.

2. How serious is the problem? Why does it matter?

Iron nutrition is a double-edged sword. While adequate iron nutriture in infants, children, and childbearing age women is essential for cognitive development and work capacity, excessive body iron in men and postmenopausal women is hypothesized to increase oxidative stress and the risk of chronic diseases such as heart disease and cancer. Research on dietary iron bioavailability provides information for policy decisions about recommended dietary allowances, nutrient supplementation, food enrichment and fortification standards, and dietary guidelines for the public. Results from this research may suggest that red meat is a good source of iron for persons with inadequate iron nutriture, without providing excessive iron for those with adequate iron stores.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 107, Human Nutrition Requirements, Food Composition, and Intake (100%)

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0401160 Year: 99 Project Number: 5450-51000-021-03 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

This research is directly related to one of ten current nutrition problems and related objectives designated in the ARS National Programs. Specifically, this research concerns the problem, "the bioavailability of nutrients in food".

4. What were the most significant accomplishments this past year?

Iron intakes in the US have increased because of increased fortification of foods and use of nutrient supplements. To help understand how well the body adapts to changes in iron intake, we investigated how well men and women adapted to supplementation with 50 mg iron daily for 12 weeks. An increase in iron stores with supplementation persisted for 6 months only in men and women with iron stores that were already adequate to high, but not in those with low iron stores. These results indicate that people with adequate iron stores do not fully adapt to prevent increased iron stores with supplementation, but that women with low iron stores may need continuing iron supplementation to counterbalance high rates of iron excretion.
Intestinal ferritin production, as measured in fecal samples, may indicate how the body adapts to adjust iron absorption. Fecal ferritin excretion was measured in iron supplemented and control subjects. The results provide new information on the variability of ferritin excretion in a free-living population and indicate that the intestine rapidly increases or decreases ferritin production in response to increased or decreased dietary iron, consistent with adaptation that was observed in nonheme, but not heme iron absorption. Fecal ferritin observations will help us understand the intestinal control of iron absorption, and differences in the control of heme and nonheme iron absorption.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Determined that daily iron supplementation reduces the efficiency of nonheme, but not heme iron absorption from food. The absorption of nonheme iron from food decreased significantly ($p<0.001$) by about 36% after 12 wk of iron supplementation. The absorption of both heme and nonheme iron was inversely and logarithmically related to body iron stores, measured as serum ferritin. The finding that nonheme, but not heme iron absorption adapts in response to daily iron supplementation will be useful in assessing the ability of humans to adapt to differences in the quantity and bioavailability of dietary iron. A better understanding of adaptation will help in establishing dietary guidelines that protect against iron deficiency or excess.

6. What do you expect to accomplish, year by year, over the next 3 years?

Year 1: This project will be completed by preparing and submitting a

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Mode Code: 5450-10-00 STP Codes: 5.1.3.2 60% 5.1.3.3 40%
National Program(s): 107 100%

technical scientific paper for publication.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The new finding that people partially adapt to iron supplementation by reducing their iron absorption from food was transferred to several customer groups, including a preliminary report to the National Cattlemen's Beef Association, which partially sponsored the research, and presentations at the national Experimental Biology meeting in Washington, DC, the International BioIron meeting in Sorrento, Italy, and meetings of the North Dakota Academy of Science and the North Dakota Dietetic Association in Grand Forks, ND.

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

None.

9. Scientific Publications:

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400524 Year: 99 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Title: HUMAN MINERAL ELEMENT REQUIREMENTS AND THEIR MODIFICATION BY STRESSORS

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Dietary excesses, deficiencies and imbalances contribute to the susceptibility and severity of a number of chronic diseases of major health and economic consequence; these include coronary heart disease, some cancers, hypertension, stroke, diabetes mellitus, atherosclerosis, cataracts and osteoporosis. Subnormal mineral element nutrition has been implicated in all of these chronic diseases. Furthermore, there is evidence that mineral elements are most important in regards to maintaining health, or will produce pathology with low intakes most markedly when a nutritional, metabolic, hormonal or physiological stressor is present that enhances the need, or interferes with the utilization of the elements. However, the importance of mineral element nutrition in the promotion of health and disease prevention, and in the reduction of health care costs, and factors that affect this importance has not been fully defined. This includes the need to provide research findings to ascertain the validity of claims that magnesium is of practical concern for maintaining bone and cardiovascular health, especially when diets are low in copper and high in foods or drinks that provide high amounts of fructose; that high dietary zinc adversely affects copper metabolism; that boron, copper, zinc, and manganese status affects calcium utilization and metabolism and thus the susceptibility to osteoporosis; and that copper, manganese, selenium and zinc affect the susceptibility to cancer induced by xenobiotic substances and reactive oxygen species.
Studies with human volunteers are and will be conducted. This includes studies examining the effects of varying intakes of zinc at different intakes of dietary copper on lipid profiles, bone status indicators, and on reactive oxygen species metabolism; the determination whether low magnesium intakes in combination with stressors that apparently increases its need induces a neurogenic inflammatory response leading to oxidative damage that can lead to pathophysiology such as cardiomyopathy, migraine headaches, and abnormal central nervous system function; and whether boron supplementation of individuals with suspected low boron status improves cognitive and motor function and indicators of bone health. Also, the effect of various intakes of mineral elements, especially copper and selenium, on the cellular functions that might be indicative of cancer susceptibility will be determined on terminally differentiated colonic epithelial cells recovered from freshly passed human stools.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400524 Year: 99 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

2. How serious is the problem? Why does it matter?

Dietary factors, including trace element nutriture, are associated with 5 of the 10 leading causes of death, including coronary heart disease, certain types of cancer, stroke and atherosclerosis. An example of the importance of nutrition in chronic disease is colon cancer, which is the second leading cause of cancer mortality in the United States and the fourth most common cause of cancer mortality worldwide. It is believed that diet is the single greatest contributor to human cancer, possibly accounting for 35 to 45% of the disease. Dietary excesses, deficiencies and imbalances in trace mineral intake is one factor that can affect cancer susceptibility.
Among those diseases that are linked strongly to diet, the cost for treatment and care in the United States exceeds $200 billion per year. Among the diseases associated with subnormal mineral element nutrition, the annual economic cost is estimated to be greater than $80 billion for cardiovascular disease, and $10 billion for osteoporosis. Several mineral elements associated with these chronic diseases including boron, copper, and magnesium have been shown to be routinely low in diets in the United States. Thus, providing information about requirements and factors that affect those requirements of critical mineral elements should result in policies and programs that improve intakes of these nutrients that will result in a healthier population, decrease the burden of chronic disease, enhance the quality of life, and diminish health care expenditures.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research relates to the National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%). The research is related to the specific National Program Components of 1) Nutrient Requirements and 2) Relationships Between Diet, Genetics and Lifestyle and the Risk for Chronic Disease. The research will develop information about the effects of deficiency or imbalance of specific nutrients on biochemical, physiological and psychological functions which should facilitate their detection and prevention, and to define requirements for health and well-being. It also will establish safe and optimal intakes, and the roles in risk or prevention of diet-related disorders, of mineral elements.

4. What were the most significant accomplishments this past year?

Mineral elements are often most important for maintaining health, or will produce pathology most markedly with low intakes when a nutritional, metabolic, or physiological stressor is present that enhances the need, or interferes with the utilization of the elements. The effect of consuming a diet rich in saturated fat (animal fats tend to be more saturated)

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Accession: 0400524 Year: 99 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

compared to a diet rich in polyunsaturated fat (vegetable fats tend to be more polyunsaturated) on mineral metabolism was investigated in 11 pre-menopausal women consuming diets either low or high in dietary manganese. Consumption of the diet rich in polyunsaturated fats significantly increased plasma ionized calcium, ionized magnesium and total magnesium concentrations and significantly decreased plasma iron concentration and transferrin saturation. The findings show that changes in dietary fat can modify mineral metabolism.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Experimental evidence using human volunteers shows that low dietary intakes of magnesium induces changes in indices used to assess the susceptibility to cardiovascular and calcium metabolism disorders. The low magnesium intake induced heart rhythm abnormalities, altered cardiovascular function and energy metabolism in postmenopausal women. Because magnesium is often consumed in inadequate amounts according to dietary surveys, these findings indicate that magnesium is of practical nutritional and clinical importance in the prevention of heart disease. Calcium balance can be maintained in postmenopausal women with intakes less than 800 mg/day, but can be altered by undesirable changes in dietary magnesium, fructose and copper.
These findings show that high dietary intakes of calcium are not the complete answer to the prevention of bone loss leading to osteoporosis. Intakes of nutrients such as magnesium, boron, copper and fructose must be appropriate for maintaining healthy bones. That is, if these nutrients are consumed in appropriate amounts, high dietary calcium intakes difficult to achieve by diet alone are unnecessary to prevent bone loss in postmenopausal women.
The consumption of high fructose decreases calcium balance with the effect more marked when dietary magnesium is low. This finding indicates that the consumption of high amounts of carbonated beverages sweetened with high fructose corn syrup is detrimental to the formation and maintenance of healthy strong bones.
The frequency of xenobiotic (certain chemical compounds) induced preneoplastic (precancerous) lesions (aberrant crypt foci) associated with colon cancer development was significantly increased in animals fed low dietary copper and tended to be increased in animals fed low dietary manganese and high dietary iron. Altered activities of antioxidant enzymes, known as superoxide dismutases, were significantly correlated with the number of the anatomical lesions associated colon cancer. These findings suggest that dietary alterations which affect superoxide dismutase activity affect cancer susceptibility. Furthermore, the effect of dietary copper and manganese on aberrant crypt foci formation may have practical implications because diets in the United States often contain copper and manganese in amounts less than their estimated safe and

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400524 Year: 99 Project Number: 5450-51000-022-00 D
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National Program(s): 107 100%

adequate daily dietary intakes.
High dietary zinc (53 mg per day) compared to low dietary zinc (3 mg per day) significantly decreased plasma cholesterol and lipoprotein A concentrations, and significantly increased various indicators of copper status including platelet cytochrome C oxidase activity and ceruloplasmin concentrations in post-menopausal women fed low (1 mg per day) or luxuriant (3 mg per day) copper. These findings do not support the dogma that a moderately high dietary zinc intakes adversely affect copper metabolism such that adverse effects on blood lipid profiles occur, including the amount of good (HDL) and bad (LDL) forms of cholesterol.

6. What do you expect to accomplish, year by year, over the next 3 years?

In FY 2000, a study with post-menopausal women will be conducted which will ascertain whether magnesium deprivation results in neurogenic dysfunction that induces a number of other undesirable changes including enhanced susceptibility to oxidative stress and detrimental inflammatory responses, which could lead to heart rhythm changes, hypertension, migraine headaches, sleep disorders, mood disturbances and osteoporosis. Methods will be developed for the use of isolated colonocytes to assess the effects of changes mineral element intakes on cancer susceptibility of humans. Human volunteers will be fed specific intakes of certain mineral elements and exfoliated cells will be prepared from their fecal material. Functions of these cells that might be indicative of cancer susceptibility in the whole individual will be determined.
In FY 2001, the analysis of all samples obtained from the magnesium study performed in FY 2000 will be completed. A study with post-menopausal women will be initiated which will further define the effects of high dietary zinc on copper metabolism. This study will emphasize the comparison of high dietary zinc with an intake of zinc equal to that of the Dietary Reference Intake.
In FY 2002 the zinc-copper interaction experiment will be completed. Studies using human volunteers will be initiated to determine whether the mineral elements nickel and silicon are of nutritional importance.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information about nutritional and clinical importance of the mineral elements magnesium, copper, boron, zinc, and manganese as it becomes available is routinely transferred to a variety of customers. The customers include the public through web pages of professional organizations and via the popular media, and other scientists through presentations at national and international meetings and professional publications.

ANNUAL RESEARCH PROGRESS REPORT
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Accession: 0400524 Year: 99 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

Presentation "Osteoporosis: Magnesium, Copper, and Boron - It's More Complicated Than You Think" to the Northern Colorado Dietetic Association.
An article "Don't Overcook Your Meat" published in the Grand Forks Herald on May 19, 1999.
An article "Migraines, Sleeplessness, Heart Attacks - Magnesium?" published in the Grand Forks Herald, September, 1999.
Both of the articles appearing in the Grand Forks Herald were also placed in the web site of the Grand Forks Human Nutrition Research Center.

9. Scientific Publications:

01. NIELSEN, F.H. 1998. The justification for providing dietary guidance for the nutritional intake of boron. Biol. Trace Elem. Res. 66:319-330.

02. NIELSEN, F.H. 1999. Ultratrace Minerals. pp. 283-303. IN: M.E. Shils, J.A. Olson, M. Shike and A.C. Ross (eds.) Modern Nutrition in Health and Disease, 9th Ed., Williams and Wilkins, Baltimore.

03. NIELSEN, F.H. 1999. Boron supplementation of peri-menopausal women affects boron metabolism, and indices associated with macromineral metabolism, hormonal status and immune function. J. Trace Elem. Exp. Med. 12:251-261.

04. DAVIS, C.D., FENG, Y., HEIN, D.W. and FINLEY, J.W. 1999. The chemical form of selenium influences 3,2'-dimethyl-4-aminobiphenyl-DNA adduct formation in rat colon. J. Nutr. 129:63-69.

05. DAVIS, C.D. and FENG, Y. 1999. Dietary copper, manganese and iron affect the formation of aberrant crypts in colon of rats administered 3,2'-dimethyl-4-aminobiphenyl. J. Nutr. 129:1060-1067.

06. NIELSEN, F.H. 1999. The balderdash and realities of health and performance claims for supplements as exemplified by calcium, chromium and vanadium. Proc. ND Acad. Sci. 53:78-82.

07. NIELSEN, F.H. and MILNE, D.B. 1999. Calcium balance in postmenopausal women with dietary calcium intakes between 650 and 860 mg/day. FASEB J. 13:Abstr. p. A869.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400524 Year: 99 Project Number: 5450-51000-022-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.1.3.4 50%
National Program(s): 107 100%

Publications: (Continued)

08. DAVIS, C.D. and FENG, Y. 1999. Effect of dietary copper, manganese and iron on the formation of aberrant crypts in colon of rats administered 3,2'-dimethyl-4-aminobiphenyl. FASEB J. 13:Abstr. p. A917.

09. NIELSEN, F.H. 1998. Magnesium - a mineral ... for optimal bone, brain, and cardiovascular function. Abstract Book, Eighth Medical Symposium, Research Day, Ross University School of Medicine, Dominica, West Indies.

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

UNOFFICIAL

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400048 Year: 99 Project Number: 5450-51000-022-02 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.3.1.2 50%
National Program(s): 107 100%

Title: NEW METHODS OF ASSESSMENT OF SPECIFIC SUBOPTIMAL MINERAL NUTRIENT STATUS IN HUMANS

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? Y

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The essentiality of zinc for human health and well-being is well established and the consequences of severe zinc deficiency have been documented in several populations worldwide. Conversely, chronic excess intake of zinc is capable of interfering with the uptake and metabolism of other trace elements, notably copper and iron. However, studies designed to examine the consequences of mild deficiency or excess dietary zinc, and to establish an optimal range of zinc intakes, have been impaired because methods currently available for the assessment of zinc status in humans are unsatisfactory.
A human study was performed in which the response to high dietary zinc (53 mg per day) in comparison to low dietary zinc (3 mg per day) was determined in postmenopausal women fed low (1 mg per day) or luxuriant (3 mg per day) copper. Several proposed indicators of zinc status were evaluated.

2. How serious is the problem? Why does it matter?

Zinc deficiency can result in impaired immune function, impaired wound healing, loss of taste and smell acuity, and, in children, reduced growth and cognitive function. The lack of a reliable indicator of human zinc deficiency impairs the determination of the extent of mild zinc deficiency in the population and thus its practical nutritional significance. This is unfortunate because recent studies have suggested that mild zinc deficiency may be a significant clinical problem, especially for elderly people living at home.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research relates to the National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%). The research is related to the specific National Program Components of 1) Nutrient Requirements, and 2) Relationship Between Diet, Genetics and Lifestyle for Chronic Disease. The research was performed to identify new indicators of zinc status that can be used to define dietary intakes for optimal health and well-being.

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400048 Year: 99 Project Number: 5450-51000-022-02 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.3.1.2 50%
National Program(s): 107 100%

4. What were the most significant accomplishments this past year?

Zinc is an essential trace element for human health and well-being; unfortunately, methods currently available for the assessment of zinc status in humans are unsatisfactory. Indicators of zinc status were measured in 25 healthy post-menopausal women fed diets containing either 1 or 3 mg of copper/2000 calories while dietary zinc at 3 and 53 mg/2000 calories were each fed for 90 days. Compared to when low zinc was fed, high zinc significantly increased extracellular but not erythrocyte superoxide dismutase, and high zinc in combination with low dietary copper significantly decreased amyloid precursor protein expression in platelets. These findings indicate that two potential new indicators of zinc status, namely, extracellular superoxide dismutase and platelet amyloid precursor protein expression have been identified.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

In addition to the accomplishment described in Question #4 above, a study with rats showed that neither changes in dietary zinc nor copper affected the amount of amyloid precursor protein, a substance that accumulates in the brain of Alzheimer's disease patients, in brain. Extrapolation of this animal finding to humans results in the suggestion that neither zinc nor copper supplementation exacerbates Alzheimer's disease, in contrast to the speculation they could based on in vitro findings.

6. What do you expect to accomplish, year by year, over the next 3 years?

The funds for this trust have been depleted. The final report has been accepted by the International Lead Zinc Research Organization (ILZRO), the entity that provided the funds. This CWU will be terminated. No research will be accomplished beyond FY 1999, but a publication which has been accepted for publication by the American Journal of Clinical Nutrition will appear in FY 2000. This publication will be listed in the publications of the parent CWU 5450-51000-022-00D in FY 2000.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information about zinc status indicators as it becomes available is routinely transferred to a variety of customers. ILZRO plans to provide the information in the final report to international organizations involved in setting environmental exposure standards, including that through oral intake. Research findings have been made available to other

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Accession: 0400048 Year: 99 Project Number: 5450-51000-022-02 T
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 50% 5.3.1.2 50%
National Program(s): 107 100%

scientists through presentations at national meetings and through professional publications.

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

9. Scientific Publications:

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

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ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0149894 Year: 99 Project Number: 5450-51530-004-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 25% 5.3.1.2 75%
National Program(s): 107 100%

Title: DEVELOPMENT AND EVALUATION OF METHODS FOR THE CLINICAL EVALUATION OF MINERAL NUTRITIONAL STATUS

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Although the essentiality of many trace elements such as copper and zinc is well established, there are currently no adequate and generally agreed upon clinical tests for evaluating copper, zinc, and magnesium nutritional status in humans. The lack of accurate diagnostic tests has impeded programs for determining human nutritional requirements and diagnosis of marginal trace mineral deficiencies that may play roles in the etiology of some chronic diseases, such as ischemic heart disease, osteoporosis, and adult onset diabetes.
Methods for the evaluation of copper, zinc, and magnesium nutritional status are being developed, and reference ranges are being established and critically evaluated for usefulness in studies using human volunteers. These include promising tests such as extracellular superoxide dismutase and 5' nucleotidase for zinc status, platelet cytochrome c oxidase and ceruloplasmin specific activity for copper status, and ionized and complexed serum magnesium for magnesium status.

2. How serious is the problem? Why does it matter?

Food consumption surveys have suggested that significant portions of the population have intakes of copper, zinc, or magnesium that are below the amount needed for maintaining good health. Yet wide-spread deficiencies of these nutrients are unrecognized, mainly because of the lack of clear-cut methods for detecting marginal deficiencies. Development of specific, accurate, and cost-effective tests for the measurement of nutritional status will aid clinicians in detecting nutritional deficiencies and imbalances in their early stages. This is particularly important for potentially malnourished portions of the population such as children and the elderly. Early detection of defects in trace element nutrition can result in considerable savings in the estimated cost for treatment and care of diet related diseases in the United States that exceeds $200 billion per year. Additionally, techniques developed will be applicable to studies on the human requirements and metabolism of trace elements. Knowledge gained by these studies will enable the evaluation of federal food and nutrition programs, and administration of programs that contribute to the health and well-being of people.

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Accession: 0149894 Year: 99 Project Number: 5450-51530-004-00 D
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National Program(s): 107 100%

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

Research on human nutrition requirements, food composition and intake is one of the priority ARS National Programs, number 107. In order to carry out components of National Programs such as 'Definition of marginal deficiencies or borderline deficiencies' and 'What are effective intervention strategies, specific, reliable and cost-effective methods for assessing nutritional status' are required. This need is recognized in a program component that states "Develop efficient economical methods of measuring the amount of essential nutrients consumed and the biologically active substances in human tissues and fluids to better assess the nutritional status of individuals."

4. What were the most significant accomplishments this past year?

In order to properly evaluate metabolic studies and to evaluate the nutritional status of individuals, "normal" reference ranges of nutritional status indicators need to be defined. Population-based reference ranges for many of the newer proposed indicators of copper and zinc status were defined in a healthy population of 63 women and 43 men. This will help in the interpretation of studies on the requirements and interactions of these nutrients.
A study investigating the effects of low and moderately high zinc intakes on copper metabolism in postmenopausal women demonstrated a significant ($P<0.007$) interaction between dietary copper and zinc intake on copper balance. Women were in negative copper balance when 1 mg copper per day was fed, but not when 3 mg copper was fed. An intake of 53 of zinc per day increased copper balance when 3 mg copper per day were fed, but significantly ($P<0.0001$) decreased copper balance when 1 mg of copper was fed. This suggests that 1 mg per day copper is inadequate for maintaining copper homeostasis, and that zinc may be needed for some aspects of copper metabolism when adequate copper is fed, but is detrimental to copper homeostasis during periods of marginal copper intake.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

A study investigating the interaction between dietary copper and magnesium demonstrated that serum and ultrafilterable serum magnesium were directly related to dietary magnesium; the changes were greater in the subjects who were fed a marginal copper diet than those supplemented with copper. A significant interaction between copper and magnesium affected the copper-containing enzyme superoxide dismutase in red blood cells (ESOD). ESOD was higher in the copper supplemented subjects when 100 mg magnesium per day were fed but it was not affected by copper when 300 mg magnesium per day

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National Program(s): 107 100%

were fed. The sequence in which magnesium supplements were given obscured the magnesium effects on variables such as serum magnesium, serum ionized magnesium, cholesterol, glucose and red cell superoxide dismutase; changes were greatest when placebo was fed first and were smaller or lacking when magnesium was fed first. This suggests that prior high magnesium intake and status inhibits or delays the appearance of magnesium deprivation signs. This information is of importance for developing further studies setting magnesium nutritional requirements and determining the consequences of magnesium deprivation.
A study investigating the interaction between dietary fructose and dietary magnesium showed that high dietary fructose significantly increased magnesium balance during both low and high magnesium intakes. Serum ultrafilterable and ionized magnesium were also affected by fructose and magnesium intakes; they were higher when fructose was fed and when magnesium intakes were high. High dietary fructose also depressed both calcium and phosphorus balances and increased urinary loss of phosphorus. These findings suggest that high dietary fructose adversely affects calcium and phosphorous metabolism in humans, particularly when dietary magnesium is low. This is of concern, because recent surveys indicate increased consumption of fructose-based sweeteners and that a significant portion of the population is consuming diets low in magnesium. Further studies are needed to see if a high fructose diet coupled with low dietary magnesium and marginal calcium leads to bone loss.
Plasma zinc, extracellular superoxide dismutase, erythrocyte membrane 5' nucleotidase, and bone specific alkaline phosphatase were shown to be sensitive indicators of zinc nutritional status. This knowledge will help in designing studies defining the human requirements of zinc during different life stages and for surveys evaluating the zinc status of populations at risk of chronic diseases where zinc may play a role.
An interaction between zinc and copper in postmenopausal women affected several zinc, copper, and iron responsive variables. The interaction between zinc and copper affected extracellular superoxide dismutase activity; the increase in activity between low zinc and high zinc was greatest in the low copper group. In addition, dietary copper seemed to increase the activity when 3 mg zinc/day were fed, but not when 53 mg zinc/day was fed. Copper and iron indicators that were depressed slightly when zinc supplements were fed included erythrocyte copper-zinc superoxide dismutase, ceruloplasmin specific activity (enzyme activity/immunoreactive protein), and hemoglobin. The depressions by zinc seemed greatest when one mg copper per day was fed. Other copper and iron indicators were either not affected or increased when zinc was supplemented. This may have been the result of decreased protein synthesis when 3 mg zinc/day was fed. Thus, no marked adverse effect of 53 mg of zinc per day in the diet on copper and iron nutriture could be demonstrated. Thus, zinc intake of this magnitude may not be as detrimental as feared on copper metabolism if copper intake is close to adequate.

6. What do you expect to accomplish, year by year, over the next 3 years?

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Accession: 0149894 Year: 99 Project Number: 5450-51530-004-00 D
Mode Code: 5450-10-00 STP Codes: 5.1.3.3 25% 5.3.1.2 75%
National Program(s): 107 100%

Population-based reference ranges will be established for many of the more newly defined indicators of copper and zinc nutritional status. Men and women whose blood cell copper-containing enzymes are in the lower 20 percentile of the population will be supplemented with copper or a placebo to determine the sensitivity of these indicators in predicting low copper status and to determine if marginal copper deficiency is a problem in this population.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Consultant on a study "Copper, zinc, manganese and bone health in postmenopausal women being conducted at the University of Georgia.
Provided methods relating to the assessment of copper, zinc, and manganese and provided advice on experimental design and best methods to use.
Provided copies of procedures detailing methods on blood cell separations and analytical procedures developed in this laboratory to other ARS scientists and investigators in other laboratories around the world.
Article "Don't let pop take fizz out of your bones" Grand Forks Herald, Feb 17, 1999.
Invited presentation: "Copper deprivation and balance" at a special forum on Recommended Dietary Intakes, sponsored by the Food and Nutrition Board. April 1999.

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

Invited presentation: "Insights on human copper requirements and indicators of copper status" to the Foods and Nutrition Department, University of GA.

9. Scientific Publications:
01. MILNE, D.B. and DAVIS, C.D. 1999. Effects of low and moderately high zinc on zinc, copper and iron nutriture. FASEB J. 13: Abstr. p. A568.

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

UNOFFICIAL

MINERAL NUTRIENT FUNCTIONS

MANAGEMENT UNIT

ANNUAL RESEARCH PROGRESS REPORT
Report of Progress (AD-421)

Accession: 0400186 Year: 99 Project Number: 5450-51000-018-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.1.3.4 25%
National Program(s): 107 100%

Title: DIETARY TRACE ELEMENTS AND PHYSIOLOGY OF THE CARDIOVASCULAR AND RELATED SYSTEMS

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The cardiovascular system (heart and circulation) is a target for biochemical deficit and structural damage in dietary copper deficiency. Although progress is being made in identifying defects in cardiovascular physiology in copper deficiency, the bases or mechanisms for these functional changes are not completely clear. The known functional changes are not related in a clear fashion to the known morphological and biochemical changes. Additionally, because most of the studies on trace element deficiencies, in particular that of copper, have not been done in humans or with dietary trace element intakes consistent with human consumption, the relationship of such findings to human health is not clear. The approaches to resolving this problem include:
A. Determination of functional changes in blood vessels and, in particular, clarification of changes in signal transduction pathways in smooth muscle and endothelium caused by dietary copper deficiency. Relevant studies will be performed with isolated vessels, on isolated organs and on whole animals. The ultimate goal is to determine the contribution of adequate copper nutrition to maintenance of blood flow to organs and to maintenance of blood pressure.
B. Identification of functional changes in the heart and their relationship to metabolic and biochemical alterations caused by trace element (copper) deficiencies. The focus will be to determine coronary blood vessel and cardiac muscle vulnerability to physiologic and metabolic stressors including, but not limited to, adrenaline stimulation and simulated heart attack (cessation and re-starting of blood flow to the heart). Isolated heart and whole animal models will be used.
C. Elucidation of general biochemical mechanisms of damage caused by copper deficiency. Oxidative stress continues to be a strong, although somewhat equivocal, candidate as a mechanism for generalized damage. Damage by oxidative mechanisms will be compared with that caused by another mechanism, glycation. The aim is to attempt to relate known enzymatic, metabolic and hormonal changes to the deterioration of function that occurs in copper deficiency. Various organs will be tested, but the primary focus will be on the heart and blood.

2. How serious is the problem? Why does it matter?

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Accession: 0400186 Year: 99 Project Number: 5450-51000-018-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.1.3.4 25%
National Program(s): 107 100%

Prior studies have indicated that dietary copper deficiency has considerable potential for contributing to chronic disease (for example, ischemic heart disease, atherosclerosis, high blood pressure) and the debilitating effects of aging. Experimental evidence indicates that a third or more of the American population may be consuming less than the Estimated Safe and Adequate Dietary Intake of copper set by the Food and Nutrition Board of the National Academy of Sciences.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

These studies directly contribute to National Program 107, Human Nutrition Requirements, Food Composition, and Intake. By examining the effect of reduced dietary copper intake on biochemical relationships in the heart and blood vessels, the subsequent effects those changes have on heart and blood vessel function and the parallels those changes have to diseases such as diabetes, aging and heart disease, these studies directly address the specific Program Components "Studies of trace element nutrition -- Biochemical and health consequences of suboptimal trace element intakes" and "Evaluations of the role of diet in affecting risk factors for disease in humans -- Abilities of dietary trace elements to reduce risk of cardiovascular disease."

4. What were the most significant accomplishments this past year?

A prior study has shown that the concentrations of two important chemical messengers, nitric oxide and cyclic GMP, are elevated in copper-deficient hearts, which suggests their possible role in reduction of heart contractile force in copper deficiency. A follow-up study this past year has shown that the amount of one of the enzymes that produce nitric oxide, inducible nitric oxide synthase, is also elevated in copper deficiency, which suggests an elevated genetic expression of this enzyme. These studies help to define the molecular basis for impaired heart function when dietary copper is restricted, provide a basis for research for scientists studying heart function in copper deficiency and illustrate to the public the dependence of good cardiovascular health upon adequate dietary copper.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Strong evidence was found supporting the view that glycation, the undesirable binding of sugar to proteins, is enhanced in dietary copper deficiency. Blood analysis revealed the presence of glycated hemoglobin and fructosamine (blood proteins with sugar bound to them) as well as pentosidine (a product of blood protein damaged by glycation). Because glycation is a process that is increased in diabetes and aging, this

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Report of Progress (AD-421)

Accession: 0400186 Year: 99 Project Number: 5450-51000-018-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.1.3.4 25%
National Program(s): 107 100%

finding suggests that reduced copper intake may worsen the consequences of these two conditions.
Measurements of heart and blood vessel function in copper deficient animals helped to show that, although cardiac output was not altered by copper deficiency, blood vessel resistance was reduced and volume of blood ejected per beat (stroke volume) of the heart was elevated; the higher stroke volume may contribute to the pathologically greater size of copper-deficient hearts. These and succeeding physiological measurements will help to characterize heart function in dietary copper deficiency.
Studies with collaborators have continued to examine the effect of copper deficiency on blood clotting function. The most recent findings have shown that the aggregation of blood platelets to one another was increased and that adhesion of platelets to blood vessel endothelial cells was reduced. Further, these findings were associated with an alteration of two platelet clotting factors, fibrinogen and von Willebrand factor. These studies emphasize the importance of dietary copper to prevent bleeding.
Another collaborative study found that the dilation of blood vessels in response to an inflammatory agent was exaggerated in copper-deficient rats. By use of appropriate blocking agents, the potential mechanism(s) responsible for this change were delineated. This study shows the importance of proper copper intake in mediating the body's inflammatory response to injury.

6. What do you expect to accomplish, year by year, over the next 3 years?

Over the next year we will determine whether and to what extent the nitric oxide signal transduction pathway is involved in the altered mitochondrial respiration of copper-deficient hearts; this will include examination of the genetic expression of the enzymes that produce nitric oxide. Research in the second year will be directed toward determining whether a direct association can be made between formation of advanced glycation end-products and altered heart function of copper deficiency. Because a positive finding in the latter study would suggest altered carbohydrate metabolism, the third year would involve studies examining the role of the pancreas in initiating heart defects of dietary copper deficiency.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Findings of the described studies have been disseminated to the general public through an article written for the nutrition section of the local newspaper and by information provided on the laboratory's internet website. Presentations describing the findings have been made to scientists at two national and two international meetings.

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8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

Invited lecture on cardiovascular effects of dietary copper deficiency at the Northern Ireland Center for Diet and Health, Coleraine, Northern Ireland, April, 1999.
Research on glycation featured in Agricultural Research magazine in an article entitled, "Can Copper Status Affect Aging?", August, 1999.

9. Scientific Publications:

01. LOMINADZE, D.G., SAARI, J.T., MILLER, F.N., CATALFAMO, J.L. and SCHUSCHKE, D.A. 1999. In vitro platelet adhesion to endothelial cells at low shear rates during copper deficiency in rats. J. Trace Elem. Exp. Med. 12:25-36.

02. SAARI, J.T. and DAHLEN, G.M. 1999. Early and advanced glycation end-products are increased in dietary copper deficiency. J. Nutr. Biochem. 10:210-214.

03. KANG, Y.J., LI, G. and SAARI, J.T. 1999. Metallothionein inhibits myocardial ischemia-reperfusion injury in mouse heart. Am. J. Physiol. 276:H993-H997.

04. SAARI, J.T., STINNETT, H.O. and DAHLEN, G.M. 1999. Cardiovascular measurements relevant to heart size in copper-deficient rats. J. Trace Elem. Med. Biol. 13:27-33.

05. WU, H-Y., SAARI, J.T. and KANG, Y.J. 1998. Inhibition of copper deficiency-induced heart hypertrophy by metallothionein in mouse. Free Rad. Biol. Med. 25:Abstr. p. S41.

06. SAARI, J.T. and BODE, A.M. 1999. Expression of inducible nitric oxide synthase is elevated in hearts of copper-deficient rats. FASEB J. 13:Abstr. p. A371.

07. SCHUSCHKE, D., FALCONE, J., SAARI, J., FLEMING, J., PERCIVAL, S. and MILLER, F. 1999. Acetylcholine-induced endothelial cell calcium ... is reduced in copper-deficient rats. FASEB J. 13:Abstr. p. A372.

08. KANG, Y.J., WU, H-Y. and SAARI, J.T. 1999. Suppression of copper deficiency-induced hypertrophy in metallothionein overexpressing transgenic mouse hearts. FASEB J. 13:Abstr. p. A371.

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National Program(s): 107 100%

Publications: (Continued)

09. SAARI, J.T. and DAHLEN, G.M. 1999. Dietary copper deficiency causes elevation of early and advanced glycation end-products. 10th Int. Symp. Trace Elem. Man Anim. (TEMA-10) Abstracts, p. 114.

10. SAARI, J.T. 1999. Copper deficiency and cardiovascular disease: Role of peroxidation, glycation and nitration. 42nd Ann. Mtg. Can. Fed. Biol. Soc. Proc., Abstr. p. 84.

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

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Accession: 0400106 Year: 99 Project Number: 5450-51000-019-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 90% 5.1.4.3 10%
National Program(s): 107 100%

Title: MINERAL ELEMENT NUTRITION, NEUROPSYCHOLOGICAL FUNCTION AND BEHAVIOR

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Behavior is unique as a criterion for establishing nutritional adequacy because it represents the functional integration of all biological systems, including compensatory mechanisms that often determine the practical importance of a nutritional deficit or excess. However, increased knowledge and a better understanding of the relationships among behavior, neuropsychological function and mineral element nutrition is required before making recommendations for mineral intakes that will facilitate optimal neuropsychological health and performance. Neuropsychological and behavioral consequences of mild and moderate deficiencies in biologically essential mineral elements are determined with the goal of improving health, work and school performance, and sense of well-being in the population. Specifically, studies are designed to determine: the role of mineral elements in cognition (i.e., attention, perception, learning, memory and reasoning) and spatial and motor skills; the effect of mineral nutrition on mood states and emotional and social adjustment; the impact on nutrition-behavior relationships of potential mediating factors, including environmental and endogenous stressors like noise, temperature, sleep duration and quality, and menstrual and menopausal symptoms; and, the effect of mineral nutrition on electrophysiology indexing brain function to gain insights into the mechanisms for nutritional effects on performance and sense of well-being. New methods and technologies are developed to increase efficacy of behavioral assessments and promote their use by other nutrition scientists. Studies of healthy adults and children are complemented by animal studies.

2. How serious is the problem? Why does it matter?

Findings obtained during the past 40 years indicate that the mineral elements boron, copper, iodine, iron, magnesium, manganese, selenium and zinc likely are important for normal neuropsychological function and behavior of adults and children. However, previous studies have yet to establish the reliability of effects of graded mineral intakes on behavior or to adequately characterize the relationship between mineral element nutrition and brain function and cognition. Such information is critical to characterizing the mechanisms responsible and is needed to apply findings to real-world problems. To respond to public interest in the

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relationship between nutrition and performance, and potentially to improve public health, productivity and sense of well-being, there is a great need to increase our knowledge of the functional consequences of graded intakes of mineral elements, and especially the consequences of marginal intakes common in many segments of the population. Food consumption surveys indicate that intakes of calcium, copper, iron, magnesium and zinc are significantly below the RDA or ESADDI for large segments of the adult population in the United States and worldwide, and many reviews have concluded that mild-to-marginal deficiencies in these and other mineral elements are particularly likely in the groups targeted by this research (e.g., women, children, elderly). Further, increased knowledge of the relationship between mineral element nutrition, neuropsychological function and behavior is needed for a more complete determination of nutrient requirements, establishing recommended dietary intakes, and evaluating the efficacy and adverse effects of taking dietary supplements, a multi-billion dollar industry in the United States.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%).
The determination of dietary requirements for optimal cognitive function and performance has been identified as a national need. This research furthers ARS objectives by directly evaluating, under highly controlled conditions, the effects of mineral element nutrition on neuropsychological function and behavior of adults, including the elderly, and in children and adolescents. Examining the combined effects of nutritional insults and exogenous and endogenous stressors offers insights into ways to improve performance in work and school, and in other situations with a high demand. Understanding the true role of mineral element nutrition in neuropsychological function and behavior also helps individuals and groups to more knowledgeably evaluate nutrition claims, and promotes healthy and cost-efficient dietary selections.

4. What were the most significant accomplishments this past year?

Research this year demonstrated that moderately high zinc (Zn) intakes (50 mg/day) can impair short-term memory of healthy postmenopausal women eating a low copper (Cu) diet (1 mg/day) as indicated by increased intrusions during verbal recall and reduced accuracy during immediate recall of numeric sequences. These data increase our understanding of the role of Cu nutriture in cognitive function, which has received little study. The impact of this finding will be to spur additional study of the relationship between dietary Cu and cognitive function, including dementia, with the potential impact to affect recommended intakes and improve cognitive function in the elderly and other populations. Results

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also address the negative effects of high Zn intakes, known to reduce Cu absorption and affect biochemical indices of Cu status.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Determined that short-term supplementation with zinc (Zn) combined with other micronutrients may improve some aspects of cognitive function of school-aged Mexican-American children, who are at increased risk for Zn deficiency primarily because of high intakes of dietary phytate. Children (aged 6-9 years) living in Brownsville, TX were treated with a micronutrient (M) mixture containing 50% RDA or mean ESSADI vitamins and minerals (excluding iron, calcium, magnesium, phosphorus and Zn, with folate at 25% RDA), 20 mg/d Zn plus micronutrients (Zn+M), 20 mg/d iron plus micronutrients, or a placebo (P) for 10 weeks in a double-blind control trial. Compared to the other treatments and P, Zn+M improved reasoning, indicated by fewer number of trials needed to learn simple concepts. This research complements earlier work by this laboratory that found a similar treatment regimen improved cognitive and psychomotor function of Chinese children with a high incidence of Zn deficiency primarily caused by inadequate Zn intakes. Findings provide further indication that Zn supplementation may significantly benefit cognitive function in deficient populations, and eventually may be used in setting recommended intakes for Zn and formulating school meal programs. While it is known that Zn is essential for growth and early development, these data provide evidence that Zn intake is important to cognitive function in later development.
Determined that zinc (Zn) deprivation (4.5 mg/day for 2 months) affected short-term verbal memory of healthy adult men. Reaction times to recognize previously learned words was initially increased and then decreased in response to deprivation, which suggests adaptation to reduced Zn intakes. Although numerous other aspects of cognitive function and psychomotor performance were also evaluated, no evidence was found for any other effects of low dietary Zn intakes in this population. Although it has been speculated that Zn nutriture may affect cognitive function in the adult, experimental study of dietary Zn effects on cognition has been extremely rare.
Found that vitamin B12 status predicted performance on tasks measuring reasoning, short-term memory, perception and attention in Guatemalan children (aged 8-12 years). B12 deficiency is known to cause cognitive impairment in the elderly. However, despite a relatively high incidence of B12 deficiency among children in many parts of the world, no research has examined the relationship between B12 nutrition and cognitive function in children. This was the first study to document a relationship B12 status and cognitive function in children. The potential impact of these findings is to receive additional resources to experimentally establish the parameters of this relationship.

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Determined that zinc (Zn) and micronutrient supplements improved cognitive and psychomotor function of rural and urban Chinese school children. Motor skills, including manual dexterity and eye-hand coordination, visual perception, memory for simple shapes and complex objects, and reasoning were functions most affected by Zn (20 mg/day) and Zn combined with other micronutrients at 50% recommended intakes. Findings indicate that cognitive and psychomotor function, and thus school performance, may be suboptimal in the >25% of Chinese children and 6-10% of school-aged children in the United States who are Zn deficient. This was the first evidence that Zn supplementation of young children may improve cognition and psychomotor function. Findings were widely reported by the national media, followed by many inquiries from the public, private industry and granting agencies, and by invitations to speak on this research. This research led directly to collaborative studies of cognitive and psychomotor effects of Zn supplementation of Mexican-American school children (supported by grants from the Gerber Foundation and USDA National Research Initiative), of zinc and iron supplementation of young women (supported by grant from the US Army Medical Research, Development, Acquisitions and Logistics Command), and of Zn deprivation of young adult men (supported by USDA ARS Western Human Nutrition Research Center). Experimentally determined that adult male rats fed diets deficient (1 ug/g diet) or excessive (100 ug/g diet) in manganese (Mn) were generally less active than those fed adequate Mn (10 ug/g diet), and consequently, these rats engaged in fewer aggressive behaviors (attacking, biting, wrestling, aggressive contact) and displacement activities (exploration, self-grooming), and more posturing. However, rats fed diets high in Mn but low in calcium (Ca; 2500 versus 5000 ug/g diet) did show increased aggressive behavior compared to rats fed other diets. Findings provide weak support for earlier reports that Mn excess increases aggression, primarily because rats fed high Mn were less active than those fed adequate Mn. No support was found for the hypothesis that Mn deprivation is associated with increased aggression. Mn is essential for normal brain function and behavior, and there has been speculation that moderate Mn intoxication or deficiency may be associated with increases in aggressive behavior. Findings from this study expand knowledge of the functional role of dietary Mn, and its interaction with Ca, at physiological as well as toxicological concentrations. Predicted impact is redirection of future research on possible nutritional involvement in aggressive behavior to study the interaction of mineral nutrients.
Showed that dietary selenium (Se) affects mood states of healthy adults. Men in the United States with typically adequate Se intakes and fed approximately 3 times the RDA for Se for 12 weeks reported less depression and mental confusion than men fed approximately one-third the RDA. Women in New Zealand with typically low Se intakes and supplemented daily with 40 ug Se reported more energy and confidence, less hostility, and a decrease in total mood disturbance after 15 weeks. Findings indicate a novel function for dietary Se that may be used to help establish Se

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requirements for adults. Findings were widely reported by the national media, followed by numerous inquiries from other researchers and the general public. Extension of this research to assess cognitive performance and brain electrical activity in relation to Se-induced mood changes is a major component of grant proposals recently submitted to the National Cattlemen's Beef Association, North Dakota Beef Commission, and USDA ARS National Research Initiative program.
Determined in mature rats that low dietary intake for 10 weeks of either copper (Cu) (0.05 versus 6.0 ug/g) and magnesium (Mg) (50 versus 500 ug/g) was associated with an increase in generalized activity. Low Cu intake also resulted in more stereotypic behavior during presentation of an auditory stressor, whereas low Mg intake resulted in increased stereotypic behavior regardless of the presence of the stressor. Low Cu intake was associated with poorer performance on measures of learning, whereas low Mg intake was associated with poorer performance on measures of memory. Neither Cu nor Mg showed strong effects on direct measures of anxiety; however, indirect measures of stressor effects during activity monitoring and memory testing suggest that both minerals may impact emotionality. Cu and Mg are two minerals of potential relevance to behavior because of their importance in neurotransmitter metabolism and because previous studies have shown that dietary intakes of both minerals affect brain electrophysiology. Findings indicate that both Cu and Mg have functional consequences at the behavioral (and possibly emotional) level, which complement earlier findings of effects of these two minerals on brain physiology. Predicted impact is future research on neuropsychological function and behavior of humans.

6. What do you expect to accomplish, year by year, over the next 3 years?

During year 2000, research will be completed on the effects of dietary manganese intakes on mood states, cognitive performance and brain function of healthy adult women participating in a controlled metabolic unit study. Research will be completed on the effects of biochemical form of selenium supplementation on central nervous system function and behavior in mature rats.
Long-term collaborative studies will be completed investigating the effects of iron and zinc supplementation on the neuropsychological function of young women living in Galveston, TX and Dunedin, New Zealand. The effects of dietary magnesium intakes on brain electrophysiology, sleep quantity and quality, and activity levels of healthy postmenopausal women participating in a controlled metabolic unit study will be determined.
During year 2001, the relationship between zinc intakes and status, body composition, cognitive function and psychoeducational performance will be examined in children, aged 12-14 years, attending local middle schools. A mobile research laboratory will be used to make all assessments.
A supplementation study will determine the dose-response relationship between copper and iron intakes and sleep quantity and quality in healthy

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adults living in the local community.
An intervention study with Guatemalan children aged 6-9 years will determine whether supplementation with vitamin B12 will improve cognitive and psychomotor function. This research is a follow-up to a recently completed study showing a correlation between B12 status and cognitive function in this population.
An epidemiological study will examine the relationship between mineral element nutrition and incidence of disease among Native Americans served by the Aberdeen Area Indian Health Services. A mobile research laboratory will be used to make all assessments.
The nutritional and health status of local institutionalized and non-institutionalized elderly will be determined. Nutritional assessment will focus on mineral nutrition. Brain and cognitive function, mood states, sleep quantity and quality, and physical activity will also be measured and related to nutrition.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

As member of the DRI Panel on Micronutrients (Food and Nutrition Board, Institute of Medicine, National Academy of Sciences), scientific literature and expert testimony on arsenic, boron, copper, iodine, iron, manganese, molybdenum, nickel, silicon, vanadium and zinc nutrition has been reviewed and interpreted for determination of the new Dietary Reference Intakes for the U.S. population.
Conducted a statewide workshop on mineral element nutrition and behavior offered through the Interactive Video Network, North Dakota State University Continuing Education, Grand Forks ND, April 1999.
To meet the need for valid yet inexpensive and easy-to-use procedures to routinely assess the relationship between nutrition and behavior, a computer software package and associated procedures were developed to automate the administration of standardized neuropsychological tasks designed to assess a variety of cognitive processes (e.g., perception, attention, learning, memory and reasoning) and psychomotor and spatial skills. Initially designed for English speaking adults, tasks and instructions have recently been redesigned to be more image-based for use with children and non-English speaking persons. This technology and related methods represent significant contributions to research on the neuropsychological and behavioral effects of nutritional deficiencies and supplementation. Users of this technology are researchers in private industry and in state and federal governments, domestic and foreign, and include the University of Texas, Wayne State University, John Stuart Research Laboratories, USDA, ARS Western Human Nutrition Research Center, University of California at Davis, Chinese Academy of Preventative Medicine in the Peoples Republic of China, and the University of Otago in

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New Zealand. Currently in progress are 6 collaborative research projects using this technology, supported by 5 granting agencies, and involving 6 researchers in 4 countries.
Continued updates and enhancements of this technology will ensure its durability. Lack of familiarity with behavioral and computerized testing, the need for careful training of test administrators, and the lack of age- and country-specific norms are current constraints on adoption of this technology.

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

Media Coverage: Women's Depression Not Linked to Low Iron. Agriculture Research. P. 23. May, 1999.
Additional coverage of research by the popular press and industry: Arbor Nutrition Guide (internet), Flair Magazine, Food and Mood, Food and Nutrition Research Briefs, Naturally Scientific Inc, Rodale Press, Woman's World, and Women's Health Resource.
Presentations: Presentations of research approach and findings were made to the public and student groups, including:
Health Occupations class from East Grand Forks Senior High School in East Grand Forks, MN (1/27/99).
Dietetics students from St. Scholastica College in Duluth, MN (4/1/99).
USDA Office of Personnel Management and Employee Development Specialists from Washington, DC (4/28/99).
University of North Dakota Alumni Association (5/26/99).

9. Scientific Publications:

01. ALLEN, L.H., PENLAND, J.G., BOY, E., DEBAESSA, Y. and RODGERS L.M. 1999. Cognitive and neuromotor performance of Guatemalan school children with deficient, marginal ... plasma vitamin B12. FASEB J. 13:Abstr. p. A544.

02. EGGER, N.G., SANDSTEAD, H.H., PENLAND, J.G., ALCOCK, N.W., PLOTKIN, R., ROCCO, C. and ZAVALETA, A. 1999. Zinc supplementation improves growth in Mexican-American children. FASEB J. 13:Abstr. p. A246.

03. PENLAND, J.G., SANDSTEAD, H.H., EGGER, N.G., DAYAL, H.H., ALCOCK, N.W., PLOTKIN, R.A., ROCCO, C. and ZAVALETA, A. 1999. Zinc, iron and micronutrient supplementation ... children. FASEB J. 13:Abstr. p. A921.

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Title: CENTER DIRECTOR

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Mode Code: 5450-20-00 STP Codes: 5.1.3.3 80% 5.1.3.4 20%
National Program(s): 107 100%

Title: BIOCHEMICAL CONSEQUENCES OF SUBOPTIMAL DIETARY INTAKE OF TRACE ELEMENTS

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Zinc and copper are cofactors for a large number of enzymes that catalyze important biochemical reactions. The effects of low zinc and copper intakes on the in vitro activities of these enzymes is one paradigm that has been used for estimating human and animal requirements for zinc and copper. However, this paradigm has not provided sufficient information to define either explicit health effects of low zinc and copper intakes or the dietary requirements of these elements for health and optimal performance. A major reason for this insufficiency is the lack of knowledge regarding how reductions in the activities of zinc- and copper-dependent enzymes perturb cellular and organ function and the lack of knowledge regarding non-enzymatic roles of zinc and copper.
Animal models of copper and zinc deficiencies and cells cultured in media containing various concentrations of zinc and copper are used to investigate the various biochemical mechanisms underlying the functional consequences of low zinc and copper intakes and low cellular content of these trace elements. The influences of copper and zinc deprivation on the synthesis of bioactive molecules, transport mechanisms, transmembrane signaling, and mitochondrial function will be determined. Knowledge based on the descriptions of biochemical mechanisms for the functional outcomes of zinc and copper deficiencies can more precisely define the dietary requirements for zinc and copper for health and optimal performance during all stages of life in men and women.
Certain dietary nutrients such as copper and zinc interact in the gut to inhibit the absorption of each other. However, zinc affects copper absorption and utilization more than copper affects zinc. If a person consumes two to three times the requirement (RDA) of zinc, either from food or, more likely, from food supplements for an extended period, copper absorption could be reduced to such an extent that the person may develop a moderate grade copper deficiency. There are numerous brands of supplements sold in the market place that contain large amounts of zinc. At present, these zinc supplements and their contents are not regulated. Therefore, there is a need to understand the physiological effects of high zinc intakes on copper absorption and utilization. We are using a human intestinal cell model to determine the basic mechanisms involved in the copper-zinc absorption interaction. This information can be used to help set standards for zinc contents in food and food supplements, and for use in making recommendations for copper intake when dietary zinc might be in excess.

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2. How serious is the problem? Why does it matter?

Current information indicates that copper and zinc intakes for a large portion of the population are below the currently recommended amounts. In particular, lower than recommended copper intakes were found in every age-sex group surveyed in the United States. It is not known if low intakes of zinc and copper can have long-term health effects, particularly in the development of degenerative diseases of the cardiovascular and nervous systems and in fetal development. Knowledge regarding the biochemical mechanisms leading to negative health effects of low zinc and copper intakes can provide a basis for recommending dietary requirements that can slow or ameliorate the development of degenerative diseases. Dietary interventions based on this knowledge could reduce the $200 billion spent yearly in the United States for treatment of diseases that are strongly associated with diet.
More than 80% of the diets consumed in the United States do not contain the recommended allowance for copper. Therefore, an intake of a small excess of zinc could easily promote or induce a mild to moderate copper deficiency. Copper is an essential nutrient for myriad physiological and biochemical processes, many of which are involved with development and function of the nervous system in the fetus. Although zinc is also required for development, it is extremely important to know the precise balance between the two nutrients so that an excess of one can be counterbalanced by the proper intake of the other.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 107; Human Nutrition Requirements, Food Composition, and Intake (100%). Program Components: Changes in needs for nutrients throughout the life cycle; Definition of marginal or borderline deficiencies.
This research will develop information about the effects of zinc and copper deficiencies on biochemical functions that will facilitate the detection of marginal copper and zinc deficiencies and define the dietary requirements of these trace metals for health, development and optimal performance throughout the life cycle. The research will also provide information that can be used to assess the risk of chronic disease from subclinical zinc and copper deficiencies.

4. What were the most significant accomplishments this past year?

One of the significant findings this year was that physiological concentrations of zinc, i.e., concentrations similar to those in the gut after a meal, can significantly reduce the transport rate of copper across an intestinal cell monolayer, apparently by reducing the relative

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abundance of the product of a specific gene involved in copper transport. The finding will further advance the understanding of the mechanisms of absorption of the important nutrient copper, and what dietary factors affect this process.
Copper deficiency during pregnancy suppresses the expression of protein kinase C-gamma in the cerebella of female neonates to a much greater extent than in the cerebella of male neonates. Although the importance of copper to brain development has been recognized for many years, the biochemical basis for this essential function of copper is still not understood. Protein kinase C expression regulates brain development and the present findings are significant because they indicate that copper deficiency targets the cerebellum as a region of the neonatal brain in which development is impacted through the suppression of protein kinase C gamma. In addition, this is the first indication that, compared to male neonates, the brain of female neonates may be more susceptible to developmental defects associated with copper deficiency.
Low dietary copper intakes reduced the amount of protein kinase C-alpha, -delta, and -zeta in the colon of rats. Decreased expression of some isoforms of protein kinase C has been observed in primary human colon tumors and chemically-induced colon tumors in experimental animals. The current finding provides a biochemical explanation for the increased incidence of precancerous lesions in colons of copper-deficient rats when they are challenged with a carcinogen. These results identify a relationship between dietary copper and protein kinase C in reducing the risk for colon cancer.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

The effect of marginal copper deficiency during pregnancy on the expression of the alpha, beta, and gamma isoforms of protein kinase C in neonatal rat brain was examined. Copper deficiency reduced the rate of expression of the protein kinase C isoforms during the three weeks following birth and led to significant reductions of protein kinase C beta in the hypothalamus and protein kinase C gamma in the hypothalamus and cerebellum. Impact: Protein kinase C expression is a determinant of brain development. Impairment in the expression of protein kinase C isoforms may eventually explain how neurological function and intellectual development are affected in the offspring of mothers who are subclinically copper deficient during pregnancy and the perinatal period.
High concentrations of zinc in the diet, equal to two to three times the RDA that can be found in over-the-counter mineral supplements, can lower the copper status of humans and may eventually lead to signs of copper deficiency. It is hypothesized that this effect is caused by alterations in the transport of copper through the intestinal epithelial cell that is carried out by specific copper transport proteins, Menkes (MNK) and hCTR1. To study this phenomenon, a cell culture system was devised that uses an

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National Program(s): 107 100%

intestinal cell mimic derived from a human colon carcinoma cell (Caco-2), that when differentiated, has similar characteristics as an intestinal epithelial cell in the body. As predicted, moderate to high concentrations of zinc in the growth media of the cells inhibited copper transport. Western blotting analysis with a specific antibody to MNK showed that at least one transcript of the protein was reduced by moderate and high zinc concentration. In addition, the high amount of zinc in the media reduced the relative abundance of MNK mRNA, the gene product necessary for the production of the protein. Impact: This work strongly suggests that zinc affects the abundance of the two newly discovered copper transport proteins, MNK and hCTR1, and, for the first time, suggests a plausible mechanism for the initiation of low copper status in humans and animals fed excess zinc in their diets.

An intriguing observation in the field of zinc nutrition is that as the amount of zinc in the diet increases, the rate of absorption of zinc from the intestine decreases. The body seems to have some mechanism that tells it how much zinc it needs and tries to adjust accordingly. However, how this mechanism works is not known. Again, we used the Caco-2 cell in culture as a model to determine if an increase in media zinc concentration would down regulate the rate of uptake and transport of zinc. We found that when cells were grown in media containing either 5 or 25 uM zinc, the rate of uptake into the cells and transport across a monolayer from the lumenal side to the basolateral side of cells was much lower with 25 uM than with 5 uM. However, in the reverse direction, the rate of uptake and transport was much higher. Impact: For the first time, this shows that the cells are using two mechanisms to control how much zinc the body gets; 1) the potential for transport into the body is slowed, and 2) the potential for transport out of the body is increased. Apparently, this helps maintain a balance of zinc so that the body does not get overloaded. Recently, another laboratory discovered a zinc transporter protein that is thought to help transport zinc. We suggest that high zinc is down regulating the production of this transporter either at the level of transcription of the gene or at the translation step for production of the protein. Positive outcomes would give clues to the question that has intrigued the nutrition research community for decades; how does zinc regulate its own rate of absorption?

Copper deficiency increases the activities of manganese superoxide dismutase in liver and heart mitochondria and glutathione peroxidase in heart mitochondria. This finding demonstrates that mitochondria experience increased oxidative stress during copper deficiency. Furthermore, the increase in oxidative stress was accompanied by increased oxidative modifications of mitochondrial proteins. This indicates that even though manganese superoxide dismutase and glutathione peroxidase activities increased during copper deficiency, these compensatory increases in antioxidant protection were not sufficient to protect mitochondria from damage by oxygen radicals. Impact: Oxidative damage to mitochondrial components and associated mitochondrial dysfunction is a major factor that

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contributes to the development of degenerative diseases and ageing. This work identifies mitochondrial damage as a component of the biological outcomes of copper deficiency that could lead to increased risk for chronic, degenerative diseases related to poor copper status.

6. What do you expect to accomplish, year by year, over the next 3 years?

During FY2000, the effects of copper deficiency on the production of reactive oxygen species by liver and heart mitochondria will be examined. This study will test the hypothesis that low copper status is a determinant of oxygen radical production by mitochondria and that mitochondria are the main source of reactive oxygen species that cause intracellular damage during copper deficiency. If mitochondrial production of reactive oxygen species is increased by copper deficiency, it will be determined if different types of dietary carbohydrate and lipid can potentiate or ameliorate increased reactive oxygen production caused by poor copper status.
Although the chemical reactivity of oxygen radicals causes damage to many types of biomolecules, these radicals also serve as second messengers for inducing proteins that protect cells against oxidative stress. Copper deficiency increases cellular oxidative stress by increasing the burden of oxygen radicals. This increase in oxygen radicals may potentiate their second messenger roles and lead to increased expression of stress related proteins during copper deficiency. In FY 2001, we will test this hypothesis by determining if copper deprivation induces manganese superoxide dismutase in HL60 cells in response to increased oxygen radical production and whether nutritionally important antioxidants can reverse the effects of copper deprivation on manganese superoxide dismutase induction. The results of this investigation will provide a foundation in FY 2002 for investigating the relationship between copper status, antioxidants, and the gene regulation of proteins that respond to oxidative stress.
In FY 2000, studies will be designed to further characterize the effect of zinc on the physiological function of the cell membrane transporters for copper. Zinc seems to affect the relative abundance of the MNK protein and its mRNA; however, it is not known exactly how zinc might have this effect. The question to be answered, is zinc involved in transcription factors that govern mRNA production, and/or is the efficiency mRNA translation affected by zinc? Answers to these questions would significantly advance our knowledge about the regulation of transport of copper and about how cells regulate the accretion of trace elements in general.
In FY 2000, studies will be designed to further characterize the effect of zinc on the regulation of zinc transporter proteins. High zinc down regulates the rate of zinc uptake into and transport across intestinal cells. Is this because the transporter protein is not being manufactured or is the transporter itself being deactivated by its own substrate?

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peroxidase and protein oxidation in liver and heart mitochondria" by W.T. Johnson and "Physiological changes in zinc concentrations of Caco-2 cell alter the uptake and transport kinetics of zinc" by P.G. Reeves were presented to scientists at Experimental Biology 99, April 17-21, 1999, Washington, D.C.

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

"Mom's Low Copper Could Harm Newborns" Agricultural Research, March 1999. "A Good Idea During Pregnancy" Food and Nutrition Research Briefs, April 1999.

9. Scientific Publications:

01. JOHNSON, W.T. and THOMAS, A.C. 1999. Copper deprivation potentiates oxidative stress in HL-60 cell mitochondria. Proc. Exp. Biol. Med. 221:147-152.

02. JOHNSON, W.T. 1999. Copper and signal transduction: Platelets as a model to determine the role of copper in stimulus response coupling. Biofactors 10:53-59.

03. BOBILYA, D.J., REYNOLDS, J.T., FAIA, K.L., BRISKE-ANDERSON, M. and REEVES, P.G. 1999. Zinc-related metallothionein metabolism in bovine pulmonary artery endothelial cells. J. Nutr. Biochem. 10:139-145.

04. JOHNSON, W.T. 1999. Effects of copper deficiency and food restriction on cytochrome c oxidase, manganese superoxide dismutase, glutathione peroxidase ... in liver and heart mitochondria. FASEB J. 13:Abstr. p. 571.

05. REEVES, P.G. 1999. Physiological changes in zinc concentrations of Caco-2 cell alter the uptake and transport kinetics of zinc. FASEB J. 13:Abstr. p. 568.

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

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Accession: 0147990 Year: 99 Project Number: 5450-51000-023-03 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.2 70% 5.2.2.2 30%
National Program(s): 107 100%

Title: HEALTH EFFECTS AND BIOAVAILABILITY OF CADMIUM FROM SUNFLOWER SEED KERNELS:A HUMAN STUDY

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The major problem centers around the fact that some crops, sunflowers in particular, that are grown in the upper Mid-Western United States contain a higher amount of cadmium than similar crops grown in other parts of the country. Cadmium is a mineral that is toxic at relatively low intakes. Two major questions need to be answered: 1) Is the cadmium in sunflower kernels available for absorption into the body, 2) and if so, does it affect the body burden of cadmium and the health of the consumer?
We are trying to resolve the first question by actually assessing the absorption of cadmium into the body by feeding kernels containing a stable isotope of cadmium and determining the amount of unabsorbed labeled Cd coming out in the feces. The difference between intake and output in the feces is a measure of absorption. The second question is being assessed by feeding sunflower kernels containing cadmium for a long period (48) weeks and determining if parameters that indicate body accumulation of the cadmium are affected. These include cadmium concentrations in urine and red blood cells. Increased concentrations indicate that cadmium may be accumulating in the body and increasing the body burden.
An additional problem involves trade restrictions on food commodities containing cadmium. At present, the European markets import sunflower kernels only if their cadmium concentration is no higher than 0.6 mg/kg. These countries propose to lower this concentration considerably. If they do, this action will severely reduce the sale of U.S. sunflower kernels to these markets. Is this proposed restriction warranted? This can be resolved by having knowledge of the availability of cadmium from the sunflower kernels, and knowledge about the effects of long term consumption of the kernels, so that rational decisions may be made on the allowable amounts of cadmium in food commodities.

2. How serious is the problem? Why does it matter?

The seriousness of this problem is two-fold. Because cadmium is best known as a toxin, the question that is ever present -- Does the intake of sunflower kernels containing a small amount of cadmium increase the body burden of cadmium, and does this, in turn, affect the health of the individual? Secondly, because of trade restrictions placed on cadmium content of food commodities, such as sunflower kernels, by European

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Markets, is the amount and availability of cadmium in this commodity sufficient to warrant further restrictions, and a possible loss of trade by the U.S. Agricultural Community?

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 107, Human Nutrition Requirements, Food Composition, & Food Safety.
This research will allow us to establish safe and adequate intakes of cadmium for optimal health and bodily functions.

4. What were the most significant accomplishments this past year?

A two-year human feeding study was finished a year ago and the massive amounts of samples were analyzed in the past year for cadmium and many other components. The study tested the null hypothesis that long-term feeding (48 wk) of sunflower kernels to humans would not affect their cadmium status as determined by current non-invasive and relevant biological tests. The data show that cadmium status indicators were not affected by feeding the cadmium-containing sunflower kernels for this long period. Impact: The information gathered in this study will be forwarded to the Joint FAO/WHO Expert Committee on Food Additives (JECFA) meeting in June, 2000, and will provide them with a more rational approach to decision making about the allowable amounts of cadmium in sunflower kernels as well as other food commodities.
A study with rats as a model was designed to determine if marginal deficiencies in zinc, iron, and calcium would affect the absorption and body burden of cadmium. Preliminary data show that mild deprivations of iron and calcium significantly increased the amount of cadmium absorbed, and the amount deposited in the kidneys; however, moderate reductions in dietary zinc did not affect cadmium absorption. These data suggest that individuals consuming diets low in iron and calcium would absorb and accumulate more cadmium than those consuming an adequate diet. Impact: Part of this information will also be supplied to JECFA to show that cadmium toxicity during low level cadmium consumption is very dependent upon the mineral nutrient status of an individual, and is an important factor to consider when making decisions about allowable amounts of cadmium in food commodities.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Accomplishment: Over the life of the project, animal studies have shown that cadmium from a diet containing 20% sunflower kernels is less available than from one containing no kernels. Studies with human volunteers have shown that individuals who report a regular consumption of

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more than 1 ounce of kernels/wk are more likely to have higher cadmium intakes than those who report eating less than one ounce/week. However, no adverse health effects were found as a result of consuming these or higher amounts of sunflower kernels. Two human studies to assess the availability of cadmium from sunflower kernels on a long-term basis, and to test the effects of long-term controlled feeding of kernels, have shown that the intestinal absorption of cadmium from the kernels ranges from 2 to 10%, but that no increase in body burden or adverse health effects were found when subjects consume up to 9 ounces of kernels/wk for 48 weeks. Impact: The Joint FAO/WHO Expert Committee on Food Additives will meet in June of 2000 to discuss recommending limits on the amount of cadmium in certain food stuffs, including sunflower kernels. The information gathered from our studies will be used in those discussions, and will provide a more rational approach to decision making about the allowable amounts of cadmium in sunflower kernels as well as other food commodities. The International Lead/Zinc Research Organization, Inc., and The National Sunflower Association supported part of this work (approximately $32,000 and $5,000, respectively). The National Program Staff provided additional support of $140,000.

6. What do you expect to accomplish, year by year, over the next 3 years?

Most of my efforts will be centered on completing the last human and animal studies and preparing manuscripts for publication and presentation. Then the project will be closed.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The findings brought forth in this research have been presented to the Food and Drug Administration, the agricultural industry, other scientists, and have been published in scientific journals. All information is immediately available for adoption. In addition, some of the findings have been disseminated in the form of presentations at national and international scientific meetings. This year Dr. Philip Reeves attended the 31st Session of the Codex Committee on Food Additives and Contaminants, at The Hague, The Netherlands, for informal discussions of these findings with a host of international participants. Later this year, all the data pertaining to cadmium concentrations in tissues and body fluids from human and animal studies will be presented to the WHO Joint Expert Committee on Food Additives, Geneva, Switzerland, for their consideration in setting recommendations for allowable cadmium concentrations in foods.

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8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

As a result of this research, Dr. Reeves was invited to review the progress of the research at numerous meetings of the National Sunflower Association. In addition, this work was presented to the Grand Forks Human Nutrition Research Center's Focus Group, which was made up of members from many disciplines, including commodity groups, local business groups, city and university governancies, and representatives from the federal government.

9. Scientific Publications:

01. REEVES, P.G., NIELSEN, E.J. and VANDERPOOL, R.A. 1999. Cadmium consumption from sunflower kernels and its availability to human volunteers. Proc. 21st Sunflower Res. Workshop. Natl. Sunflower Assoc, ed. pp. 46-55.

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Title: CENTER DIRECTOR

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Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.2.2.2 25%
National Program(s): 107 100%

Title: BIOCHEMICAL, PHYSIOLOGICAL, AND NUTRITIONAL ROLES OF CERTAIN ULTRATRACE ELEMENTS

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:
1. What major problem or issue is being resolved and how are you resolving it?

Numerous mineral elements consumed in ultra trace amounts have been considered to be of possible importance in the prevention of disease with nutritional roots, or for the enhancement of health and longevity. Many of these elements, because of some promising physiological or clinical finding (most often in an animal model or a special human situation) are often ostentatiously promoted for the purpose of financial gain. Health and nutrition professionals often have these elements brought to their attention by clients because these promotions often claim that supplements of these mineral elements can prevent some feared diseases such as cancer, heart disease and loss of cognitive function, or can enhance physical appearance. Among these elements are those for which there is circumstantial evidence for essentiality, but are not unequivocally accepted as essential because they lack a defined specific biochemical function in higher animals. Some of these elements are inappropriately promoted (e.g., vanadium) such that intakes detrimental to health may be occurring. Other ultra trace elements apparently have health benefits that are now only being discovered or defined (e.g., boron). Therefore, credible and data-supported dietary recommendations need to be developed for some ultra trace elements so that they are consumed in amounts that assure health and well-being. Additionally, regulatory agencies will increasingly consider nutritional and health benefits of nutrients, especially minerals, so that risk assessments and toxicological standards do not conflict with amounts beneficial to health, in addition to not causing economic burdens to reduce environmental exposure to amounts that may do more harm than help in preserving health and well-being.
Animal and human experiments are and will be conducted to define the biochemical and physiological roles of various ultra trace elements, including arsenic, boron, nickel, silicon, and vanadium. The basic approach will be to feed experimental animals or human volunteers diets that contain amounts of specific ultra trace elements and other selected nutrients and non-nutrients (postulated to affect the metabolism and utilization of specific ultra trace elements) which are accurately controlled and systematically varied. The response of the animals and humans to the dietary manipulations will be ascertained by evaluating various appropriate biochemical, physiological and anatomical variables. Biochemical and molecular biological methods will be used to define exact biochemical roles of specific ultra trace elements. The findings will help in the determination of the nutritional importance of specific ultra trace

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elements and whether their dietary intake influences the occurrence of chronic disease.

2. How serious is the problem? Why does it matter?

A paradigm shift has occurred in the setting Recommended Dietary Allowances which have been renamed Dietary Reference Intakes (DRIs). The dominant role of deficiency in the determination dietary guidelines is now complemented by the total health effects of dietary components or nutrients. Thus, where sufficient data for efficacy and safety exists, the setting of new DRIs will consider reduction in the risk of chronic disease in addition to preventing deficiency pathology. This is important because dietary factors are associated with 5 of the 10 leading causes of death, including coronary heart disease, certain types of cancer, stroke and atherosclerosis. Among those diseases that are linked strongly to diet, the cost of treatment and care in the United States exceeds $200 billion per year. Recognition that nutrition is important in health promotion and disease prevention, has spawned a plethora of health enhancing foods and supplements, now often called "functional foods" or "nutraceuticals" that represent an exploding market in the United States conservatively estimated at $29 billion a year. Many of the health claims for these health enhancing foods and supplements, however, have not been substantiated by basic research and human feeding trials. Many of the health claims include the use of ultra trace elements because their intakes have been associated with one or more chronic diseases. Thus, research is needed to establish which foods and in what amounts will provide clinically important ultra trace elements in amounts that promote health and disease prevention, and to determine safe intakes of specific ultra trace elements so that the setting of reasonable toxicological standards can be accomplished. This information should result in policies and programs that will result in a healthier population, decrease the burden of chronic disease, enhance the quality of life, and diminish health care and environmental exposure protection expenditures.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research relates to the National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%). The research is related to the specific National Program Components of 1) Nutrient Requirements; and 2) Relationship Between Diet, Genetics and Lifestyle for Chronic Disease. The research will develop information about the effects of deficiency or imbalance of specific nutrients on biochemical, structural, physiological and psychological functions to facilitate their detection and prevention, and to define requirements for health and well-being throughout the life cycle. It also will establish safe and optimal intakes, and the roles in risk or prevention of diet-related chronic

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diseases, of ultra trace elements.

4. What were the most significant accomplishments this past year?

Although other laboratories have provided experimental evidence that boron is essential for the normal embryological development of lower species including the frog and zebrafish, the importance of boron for embryological development of mammals needs to be established because this is more likely to be applicable to humans. Dietary boron, fed in nutritional amounts, was found to interact with a supplemental naturally-occurring, boron-complexing agent (erythritol) to affect the number of fetal absorptions in an animal nutrition-pregnancy model system. This finding is the first to indicate a role for boron during gestation in mammals. This finding suggests that boron may be important in human reproduction.
The development of a mathematical model of nickel metabolism in rats should help show the essentiality of nickel and provide credible data to set dietary recommendations for humans. By the use of the isotope nickel-63, it was found that 2.5% of nickel ingested is absorbed and very little is retained. A model was developed which suggests that nickel is homeostatically controlled by absorption and retention processes, and that tissue nickel is metabolized by at least three different mechanisms. The model and findings provide further evidence that nickel is an essential element for laboratory animals and thus, is most likely important for human health.
There is evidence that an interaction between arsenic and copper can affect the toxicological or beneficial effects of arsenic or the need for copper. Rats fed various amounts of arsenic and copper showed that an interaction between these two elements affected many parameters including ceruloplasmin, heart weight/body weight ratio, and the concentration of trace elements in liver and kidney. The findings indicate that high dietary arsenic can exacerbate copper deficiency. Thus, copper status is important when one considers the effects of arsenic toxicity.
More objective information and creative approaches are needed to derive sound dietary recommendations for mineral elements such as zinc and iron. Novel mathematical approaches (logistic regression, discriminant analysis) were used to determine the impact of the interaction between zinc and iron on the requirements of these two elements. It was found that iron affects zinc metabolism to a much greater extent than zinc affects iron metabolism. The approaches used showed that interaction between mineral nutrients must be considered when regulatory groups set recommended dietary intakes.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Normal or nutritional dietary intakes of boron help control the normal

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inflammatory process and therefore reduce the development of inflammatory disease. In an arthritic animal model system, physiological amounts of dietary boron increased concentrations of natural killer cells and also attenuated the rate of paw swelling. These findings suggest that physiological amounts of boron reduce the risk for inflammatory disease by helping hold in check a system that is constantly poised to attack, a balance that permits pathogen elimination but avoids autoimmunity. The national cost of rheumatoid arthritis, an inflammatory disease, is similar to that estimated for heart disease and stroke. The finding that boron controls the degree of severity of a form of experimental rheumatoid arthritis suggests that dietary recommendations for boron could have an impact on the occurrence and severity of this disease.

Vanadium was found to be a nutritionally important element involved in thyroid hormone and glucose function or metabolism. However, the findings indicated that the amount of vanadium needed to assure appropriate thyroid hormone and glucose metabolism was extremely small and that supra nutritional amounts, similar to amounts provided by supplements available to people, induced changes that could be construed as not beneficial. This suggests that some over-the-counter supplements sold as anabolic or anti-diabetic agents could be detrimental to health.

Vanadium status affected the response of rats to N-nitro-L-arginine methyl ester (L-NAME), an inhibitor of nitric oxide formation. Also, when compared to vanadium-deprived rats, the response of rats to nutritional amounts of vanadium was different than when supra nutritional amounts (but not normally considered toxic) of vanadium were fed. This was most apparent in thyroid hormone status, energy metabolism, and oxidative metabolism status indicators. The findings suggest that vanadium has a biological role in addition to pharmacological activity, involving a reactive oxygen species and supports the concept that vanadium is an essential nutrient but is needed in very low amounts.

Arsenic was demonstrated to have a physiological role that affects methionine metabolism, most likely, methionine recycling. Arsenic deprivation increases liver S-adenosylhomocysteine (SAH), decreases S-adenosylmethionine (SAM), and thus, decreases the ratio of SAM to SAH. A low SAM/SAH ratio has been associated with an increased risk of certain types of cancer. These findings in addition to others obtained during the life of the project indicate that arsenic is a nutritionally essential ultratrace element whose need is influenced by nutritional stressors that affect sulfur amino acid metabolism. This accomplishment has been used to challenge the need to establish an extremely low toxicity threshold for arsenic in drinking water (i.e., decreasing the allowable concentration from 50 to as low as 5 ug/L) and food and thus save a significant (billions of dollars) cost to the public.

Fuzzy logic was first used in the derivation of nutrient requirements. Other novel mathematical approaches for determining nutrient requirements have also been proposed. An initial nonlinear model was developed to account for interactive effects of other nutrients and non-nutrients on

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requirements. These approaches are more objective and can easily be adapted as new information becomes available for the derivation of recommended intakes. Also, because they account for interactive effects (synergistic or antagonistic), the models can be tailored to a specific group of individuals.
Nickel and interactions among nickel, folic acid and vitamin B12 were found to affect the vitamin B12 and folate dependent pathway of methionine synthesis from homocysteine. The findings indicate that nickel has an essential biological role which could influence the risk factors associated with coronary heart disease of decreased circulating vitamin B12 and folic acid, and increased circulating homocysteine. Moreover, because nickel deprivation exacerbates folic acid deficiency, nickel nutriture might be a factor in some cases of neural tube defects caused by folic acid deficiency.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2000 - The effects of boron deprivation and physiological amounts of boron will be examined in post menopausal rheumatoid arthritic volunteers. The design of the study should give definitive answers as to whether low boron nutriture, not uncommon in the United States, is a factor in the prevalence and severity of rheumatoid arthritis.
Animal experiments will be completed that are designed to ascertain whether homocysteine status affects the response to nickel deprivation, or conversely, whether nickel has a function that affects homocysteine metabolism and thus is a factor in the association between elevated circulating homocysteine and the risk of heart disease. Also, animal experiments will be conducted with the objective of isolating of nickel-containing proteins that may have important biological functions.
Animals will be used to show that arsenic directly affects the cycling of methionine and that altered methionine metabolism affects arsenic deprivation. This will help direct further efforts in the determination of the exact site at which arsenic has an essential or beneficial biological effect. Animal experiments will be completed which show that arsenic deprivation affects the response to selenium deprivation and vice versa. Also, animal experiments will be conducted to confirm and extend the observation that selenium deprivation alters homocysteine metabolism.
Mathematical models will be extended and refined to show the importance of interactions among nutrients in determining the dietary requirements copper, iron and zinc, and to model nickel metabolism in nickel-deficient rats.
FY 2001 - Flow cytometry methods will be used to identify cell surface molecules that bind boron; findings should be obtained that will help elucidate the biological role of boron in mammals.
By the use of isotopic arsenic, attempts will be made to isolate arsenic-dependent proteins from animals. Whether any proteins isolated are related to methyl metabolism will be ascertained.

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Animal experiments will be conducted to ascertain whether nickel has a function at the cell membrane level affecting signal transduction involving ionized calcium and/or phosphate moieties.
A diet low in silicon will be developed that can be fed to human volunteers so it can be established that silicon is a nutritionally important element.
FY 2002 - Studies will be initiated to elucidate the induction mechanism whereby dietary boron increases the concentration of natural killer cells in the serum of animal models with experimental arthritis.
An experiment with human volunteers will be initiated to establish whether they respond to nickel deprivation in a manner similar to that of animals. Status indicators developed from animal experiments in FY 2000 and 2001 will be used. The findings should help establish the nutritional importance of nickel.
Evidence will be obtained that will establish whether arsenic deprivation affects the susceptibility to certain types of cancer, and that this susceptibility is influenced by other nutrients that affect methyl metabolism.
An experiment with human volunteers will be initiated to establish whether silicon is needed for healthy connective tissue function, including wound healing.
Mathematical approaches that use easily accessible blood variables to predict mineral status of animals and humans will be finalized. These approaches should be useful for determining mineral requirements of specific groups or individuals.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

An exhaustive synopsis of all boron nutrition research literature relevant for humans was compiled at the request of the Dietary Recommended Intake Micronutrient Panel of the Food and Nutrition Board (Institute of Medicine, National Academy of Sciences). Much of the relevant literature emanated from this CWU. The synopsis was consulted by the panel during their current consideration of dietary guidelines for boron.
Knowledge was transferred through invited presentations at the forum entitled "Dietary Reference Intakes for Arsenic, Boron, Molybdenum, Nickel, Silicon, Vanadium, and Other Trace Elements" organized by the Panel on Micronutrients, a working subcommittee of the Food and Nutrition Board (Institute of Medicine, National Academy of Sciences); these presentations included "Boron Balance and Bone Health" by Curtiss Hunt, and "Requirements for Arsenic and Impact on Human Health" by Eric Uthus. Forrest H. Nielsen presented "Requirements for Nickel and Impact on Human Health," "Requirements for Silicon and Impact on Human Health," and "Requirements for Vanadium and Impact on Human Health." The information

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National Program(s): 107 100%

presented will be used to determine the Dietary Reference Intakes (DRIs) for boron, arsenic, nickel, silicon and vanadium.
Information about the nutritional or beneficial aspects of ultra trace elements as it becomes available is routinely transferred to a variety of customers. The customers include risk assessments groups through direct contact or organized workshops; the public through web pages of professional organizations and via the popular media, and other scientists through presentations at national and international meetings and professional publications.

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

"Nickel Plays An Essential Role for Humans," an article published by the Grand Forks Herald newspaper and on the Grand Forks Human Nutrition Research Center's Home Page. (Eric Uthus)
Presentation via the North Dakota Extension Service's Interactive Video Network "Boron-A New Nutrient." (Curtiss Hunt)

9. Scientific Publications:

01. HUNT, C.D. 1998. Regulation of enzymatic activity. One possible role of dietary boron in higher animals and humans. Biol. Trace Elem. Res. 66:205-225.

02. LANOUE, L., TAUBENECK, M.W., MUNIZ, J., HANNA, L.A., STRONG, P.L., MURRAY, F.J., NIELSEN, F.H., HUNT, C.D. AND KEEN, C.L. 1998. Assessing ... boron diets ... in rodents ... systems. Biol. Trace Elem. Res. 66:271-298.

03. MEACHAM, S.L. and HUNT, C.D. 1998. Dietary boron intakes of selected populations in the United States. Biol. Trace Elem. Res. 66:65-78.

04. NIELSEN, F.H. 1998. The nutritional ... animals. pp. 297-307. IN: A.S. Tracey and D.C. Crans (eds.) Vanadium Compounds, Chemistry, Biochemistry, and Therapeutic Applications, ACS Symp. Ser. 711, ACS, Washington, DC.

05. HUNT, C.D. 1999. Dietary boron as a physiological regulator of the normal inflammatory response: a review and current research in progress. J. Trace Elem. Exp. Med. 12:221-233.

06. ZASLAVSKY, B., NIELSEN, F.H. and UTHUS, E.O. 1999. Predominant physiological factors in the response of rats to changes in dietary vanadium. Proc. ND Acad. Sci. 53:97-102.

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Publications: (Continued)

07. HUNT, C.D., IDSO, J.P. and KEEHR, K.A. 1999. Dietary boron alleviates adjuvant-induced arthritis (AIA) and changes the blood concentrations of neutrophil, CD8a, and ... cells in rats. FASEB J. 13:Abstr. p. A545.

08. UTHUS, E.O. 1999. Compartmental model of orally administered nickel (Ni) in rats. FASEB J. 13:Abstr. p. A577.

09. ZASLAVSKY, B. and UTHUS, E.O. 1999. Prediction of dietary copper, zinc and iron based on response factors in blood and plasma of rats. FASEB J. 13:Abstr. p. A535.

10. HUNT, C.D. 1999. Dietary boron is a physiological regulator of the normal inflammatory response. Tenth International Symposium on Trace Elements in Man and Animals, TEMA-10, Program and Abstracts, p. 100.

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

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Accession: 0401535 Year: 99 Project Number: 5450-51520-011-03 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 50% 5.2.2.2 50%
National Program(s): 107 100%

Title: THE NUTRITIONAL ROLE OF BORON IN THE INHIBITION OF SERINE PROTEASES

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

The normal inflammatory response serves to focus host defenses at a site of tissue injury or infection. Thus, under normal conditions, the defense mechanism of inflammation serves a vital function. Typically, elimination of antigens proceeds without evidence of clinically detectable inflammation. Excessive inflammation leads to inflammatory disease (for example, rheumatoid arthritis). This can occur when the immune system has encountered either an unusually large amount of antigen, antigen in an unusual location, or antigen that was difficult to destroy. As first shown in this laboratory, dietary boron reduces the onset and severity of induced arthritis in rats. Also, certain boron compounds are potent in vitro inhibitors of several enzymes that regulate the normal inflammatory reaction. Therefore, the focus of this project is to identify inflammatory mediators that interact with boron. This approach will help establish the specific function of boron in humans and subsequently be useful in identifying marginal or borderline boron deficiency.
Based on molecular structure, several classes of compounds predicted to interact with boron were selected and assessed to determine the exact role of boron in regulation of the inflammatory response. The basic approach will be to determine the in vitro binding affinities of these compounds to boron. Compounds with physiologically significant boron binding affinities will be investigated as potential indicators of boron status by in vivo measurement of these compounds after extraction from tissues of animals fed boron deficient diets.

2. How serious is the problem? Why does it matter?

A paradigm shift has occurred in the setting of the Recommended Dietary Allowances which have been renamed the Dietary Reference Intakes (DRIs). The new DRI values reflect the latest understanding about nutrient requirements based on optimizing health in individuals and groups, not just the prevention of nutrient deficiency. Therefore, establishing the new DRIs will involve consideration of the effect of the nutrient on reduction in the risk of chronic and other diseases and conditions. Inflammatory diseases cost Americans billions of dollars yearly in treatment and loss of productivity. Rheumatoid arthritis in particular is a painful, chronic, recurrent, systemic inflammatory disease that affects

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1-3 percent of Americans. Thus, prevention or significant amelioration of inflammatory diseases including rheumatoid arthritis by relatively simple dietary means, would have significant impact. There is a high probability that normal amounts of dietary boron will significantly ameliorate symptoms of rheumatoid arthritis in humans based on its known effect on cartilage development and maintenance and influence on the progression of experimental rheumatoid arthritis in animal model systems. Identification of biomolecules that interact directly with boron will serve to define borderline boron deficiency and develop indicators of boron deficiency.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research relates to the National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%). The research is related to the specific National Program Component of definition of marginal deficiencies or borderline deficiencies. The research is especially suited to defining borderline boron deficiency and to develop indicators of boron deficiency.

4. What were the most significant accomplishments this past year?

Identification of biomolecules that interact directly with boron will serve to define borderline boron deficiency and develop indicators of boron deficiency. Scientists in this laboratory grew cells, isolated from an animal, in a nutrient solution and measured the amount of boron that was transferred from the outside to the inside of the cells. They discovered that cells of the immune system grown in a normal culture medium internalize boron such that the concentration of the element was twice as high on the inside compared to that on the outside of the cells. Because boron is retained within animal model cells, it is reasonable to hypothesize that there is an uncharacterized intra-cellular molecule that either binds or incorporates boron, a finding that provides more indirect evidence for an essential role for boron in animal nutrition models and humans.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Scientists in this laboratory have developed and implemented an in vitro model system that determines the direct binding of boron to biomolecules. The method utilizes capillary electrophoresis technology and allows for the discrete separation and identification of biomolecules that bind to boron and also allows for discrimination of binding interactions. When molecules are separated by capillary electrophoresis in the absence or presence of boron, the degree of boron binding is indicated as a change in migration time of the molecule through the solution. An increase in

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migration time indicates increased boron binding. Various biomolecules can be compared for their ability to bind boron. The information may be used to determine the exact biological role of boron in humans.
The new capillary electrophoresis method was used to demonstrate the direct binding of boron to biomolecules of considerable physiological importance including the signal hormone, diadenosine tetra-phosphate. As predicted, the migration time of this compound was increased as concentrations of boron were increased. The increase in migration time indicates that boron binds to this important molecule that controls cell growth and the response of the cell to stress. The information may be used to help find the exact biological role of boron in the control of inflammatory diseases including rheumatoid arthritis.
The finding that cells isolated from a mouse monocyte-macrophage cell line and grown in a normal culture medium internalize boron provides additional indirect evidence for an essential role for boron in animals and humans.

6. What do you expect to accomplish, year by year, over the next 3 years?

During FY 2000, the capillary electrophoresis method developed in this laboratory will be used to examine interactions between boron and cellular molecules. A series of in vitro experiments will be conducted to further characterize the interaction between boron and ribonucleoside phosphates with special emphasis on diadenosine tetra-phosphate that is known to act as a signal hormone to regulate cell proliferation and stress responses. During FY 2001, experiments will be conducted to determine inflammatory cell concentrations of relevant ribonucleosides in boron-deprived experimental animal models. During FY 2002, experiments will be conducted to determine the interaction between boron and serine proteases at physiological concentrations in animal models of human nutrition because boron is known to inhibit serine protease activity in vitro.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

The capillary electrophoresis method for determining and evaluating the interaction between boron and biomolecules was described and discussed at an international meeting organized and sponsored by a prominent international company that provides various boron-based products on a world-wide basis.

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

None.

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National Program(s): 107 100%

9. Scientific Publications:
01. RALSTON, N.V.C. and HUNT, C.D. 1999. Evidence for mono- and dicomplex boroester formation in biomolecules obtained from capillary zone electrophoresis (CE) binding studies. FASEB J. 13:Abstr. p. A545.

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

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Accession: 0402077 Year: 99 Project Number: 5450-51520-011-04 T
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 80% 5.2.2.2 20%
National Program(s): 107 100%

Title: DETERMINATION OF THE ESSENTIALITY OF NICKEL

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Numerous mineral elements consumed in ultra trace amounts have been considered to be of possible nutritional importance in the prevention of disease with nutritional roots, or for the enhancement of health and longevity. Among these elements are those for which there is circumstantial evidence for essentiality, but are not unequivocally accepted as essential because they lack a defined specific biochemical function; nickel is one these elements. Because defined biochemical functions have been identified in lower forms of life, it is likely nickel has an essential biochemical function in higher forms of life including humans. There is a need for this biochemical function to be identified. Experiments will be conducted with rats and possibly chicks to identify a metabolic pathway that is influenced by dietary nickel. Initially, these experiments will be factorially arranged with low and luxuriant amounts of nickel fed in diets formulated to alter certain specific pathways by the inclusion of nutritional stressors. Once a pathway influenced by nickel is identified, biochemical and molecular biological methods will be used to define the exact locus where nickel has an essential function. This function might be useful as a status indicator; if so, the response of human volunteers to a nickel low diet will be ascertained.

2. How serious is the problem? Why does it matter?

Lack of unequivocal acceptance of essentiality inhibits the consideration of the possible health benefits of nickel when regulatory agencies develop risk assessments and toxicological standards for this element. Thus, these standards might conflict with amounts of nickel people should consume because they are beneficial to health, and could cause economic burdens to unnecessarily reduce exposure to this element. Moreover, the lack of a status indicator for nickel inhibits the determination of the nutritional importance, and prevents the provision of solid dietary, guidance for this element.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

The research relates to the National Program 107, Human Nutrition

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National Program(s): 107 100%

Requirements, Food Composition and Intake (100%). The research is related to specific National Program Component of Nutrient Requirements. The research will develop information about the effects of nickel deficiency, and help establish safe and optimal intakes of nickel that will promote health and well-being throughout the life cycle.

4. What were the most significant accomplishments this past year?

This project was initiated this year. The Research Associate employed to conduct the research of this project entered duty in March 1999. He is currently completing an experiment with rats to ascertain whether there is a relationship between nickel and homocysteine metabolism. Preliminary evidence suggests that the response to nickel deprivation is affected by changes in dietary pyridoxine and sulfur amino acids which affect homocysteine metabolism. The findings might give possible insight to a biological function for nickel.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

See the answer to Question # 4.

6. What do you expect to accomplish, year by year, over the next 3 years?

Funds for this project have been provided for 2 years only.
FY 2000 - Experiments will be completed that will determine the relationship between nickel and homocysteine metabolism. Experiments will also be done that will help determine the mechanism through which dietary nickel affects nitrogen metabolism and plasma lipids.
FY 2001 - Findings obtained in FY 2000 should identify a possible nickel function that can be confirmed and defined. If this function is one that can be related to a blood parameter, a preliminary study with humans will be initiated to see if the parameter varies with nickel intakes that commonly occur and whether the variation would have any nutritional implications.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

Information about nickel essentiality as it becomes available is routinely transferred to a variety of customers. The first formal reports of findings emanating from this project will occur early next year at professional meetings.

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8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

9. Scientific Publications:

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

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Accession: 0400356 Year: 99 Project Number: 5450-51530-003-00 D
Mode Code: 5450-20-00 STP Codes: 5.1.3.3 75% 5.3.1.1 25%
National Program(s): 107 100%

Title: MINERAL ELEMENTS, PHYSIOLOGICAL FUNCTION & PERFORMANCE AND BODY COMPOSITION

Period Covered From: 01/99 To: 09/99

Would you like to terminate this Project? N

Progress and Outcomes:

1. What major problem or issue is being resolved and how are you resolving it?

Recommendations for the dietary intake of mineral elements, with an emphasis on zinc, copper and chromium, based on the promotion of health and optimal biological function are generally lacking. Studies designed to examine the effects of restricted dietary intakes of mineral elements on physiological function are needed to ascertain appropriate amounts of mineral elements in the diet to maintain health and to facilitate the attainment of genetic potential of biological functions. One consideration in delineating appropriate dietary mineral intakes is assessment of food-borne factors that affect the absorption and utilization of dietary minerals. Also, the use of environmental stressors (i.e., controlled exercise and cold/hot temperatures) is another factor used to determine mineral element needs of physically active people.

Studies are conducted in animals and humans. Graded intakes of dietary zinc and copper are fed and physiological functions are monitored to delineate intake amounts that affect physiological function with an emphasis on energy utilization, cardiorespiratory function, work performance, and heat production. Studies of human volunteers fed whole food diets low in chromium and supplemented with specific chromium compounds and other compounds hypothesized to influence the absorption and utilization of chromium and other minerals are conducted. Other studies are undertaken in which animals are fed a diet low in iron, then given diets containing varied amounts of iron (low and adequate) and different types (saturated and polyunsaturated) of dietary fat. Changes in iron status and hematology, as well as changes in bone mineral content, are determined.

2. How serious is the problem? Why does it matter?

There is considerable debate regarding the amount of dietary copper, zinc and chromium required for health maintenance and optimal biological function. Previous approaches focused on relatively insensitive measures of nutritional adequacy (chemical balance). By relating dietary mineral intakes to measurements of biological function, such as energy utilization, heart rate and blood pressure, work production, heat generation, and glucose and lipid metabolism, suggestions for dietary mineral intakes are made in reference to quality and quantity of life.

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Much of this research is requested by physically active individuals who seek to optimize physiological function without the use of dietary supplements and health managers who seek to minimize health care costs of the American public.
Iron deficiency is the single most prevalent nutritional deficiency in the world. Attempts to fortify food products have been unsuccessful in ameliorating this pervasive nutritional problem among females. In parallel with the incidence of iron deficiency, dietary fat consumption patterns indicate an increase of polyunsaturated fat intake. Studies indicate that the type of dietary fat affects iron, specifically non-heme iron, absorption and utilization. Polyunsaturated fat reduces and saturated fat, specifically stearic acid, promotes iron utilization. Because stearic acid is neutral to serum cholesterol and lipoprotein cholesterol concentrations, it offers practical benefits in ameliorating iron deficiency in animals and humans.

3. How does it relate to the National Program(s) and National Program Component(s) to which it has been assigned?

National Program 107, Human Nutrition Requirements, Food Composition and Intake (100%). The specific Program Components include Definition of Marginal or Borderline Deficiencies - Biochemical and Health Consequences of Suboptimal Trace Element Intake, Methods of Nutritional Status Assessment, and Function and Metabolism of Nutrients for Physical Development.
This research will acquire information about the effects of graded trace element deficiencies, emphasizing zinc, copper, magnesium, iron, and chromium, on biochemical measurements and physiological functions. This information will facilitate the detection of marginal mineral deficiencies and define dietary requirements of these mineral elements for the development and maintenance of health and optimal function throughout the life cycle. The research will provide needed information that can be used to assess the risk of chronic diseases and impairments in subtle physiological functions that arise from mild and moderate mineral element deficiencies.

4. What were the most significant accomplishments this past year?

Simple, non-invasive methods, which are accurate and capable of reliably determining changes, to assess human nutritional status, particularly muscle mass, are lacking. We conducted a pilot study in which we adapted the bioelectrical impedance method, that was developed at the Grand Forks Human Nutrition Research Center, for the assessment of muscle mass in the upper legs (thighs) of overweight women who participated in a controlled weight loss program. Preliminary results showed that the bioimpedance determinations of muscle were similar to the reference measurements made with dual x-ray absorptiometry. These initial findings indicate that this

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new method is a valid and accurate method for routine determination of muscle mass in humans; this impedance approach also was a better indicator of muscle mass than the currently used measures of thigh circumference to estimate muscle mass.
Assessment of zinc nutritional status is complicated by the availability of blood biochemical markers that clearly distinguish between adequate and deficient zinc status and differentiate between degrees of zinc status, marginal and severe deficiency states. We evaluated the response of a zinc-containing enzyme in plasma, extracellular superoxide dismutase, in a series of studies of rats fed different amounts of dietary zinc and zinc-deficient rats supplemented with zinc. We found that the activity of this enzyme was more responsive to differences in dietary zinc, particularly in the rehabilitation of the zinc-deficient rats, than the currently used biochemical indicators of zinc status, including plasma zinc. These findings suggest that extracellular zinc superoxide dismutase activity is a sensitive and specific marker of zinc status that may be useful in assessing human zinc nutritional status among individuals in national nutritional surveys.

5. Describe the major accomplishments over the life of the project, including their predicted or actual impact.

Restricted dietary zinc adversely affected energy utilization during exercise in young men. As compared to a dietary zinc intake of 18 mg/d, 3 mg zinc daily was associated with significant alterations in energy production and respiratory function during progressive peak exercise on a cycle ergometer. The mechanism of this impairment was a significant decrease in red blood cell carbonic anhydrase activity with restricted dietary zinc. Carbonic anhydrase is a zinc-containing enzyme with the specific function of transporting carbon dioxide from cells to the lungs for excretion. Zinc deficiency was confirmed with a significant loss of zinc, negative zinc balance, and decreased serum zinc concentration when dietary zinc was restricted. Impact: These findings provide the first evidence of impaired physiological function when dietary zinc is fed in an amount similar to that consumed by some physically active individuals. Also, the finding of decreased carbonic anhydrase activity in the red blood cell in response to low dietary zinc suggests that the activity of this zinc-containing enzyme may be a new blood biochemical marker for assessment of human zinc nutritional status.
Blood cells are not an appropriate tissue for determination of chromium nutritional status. A pilot study revealed that the chromium concentrations of populations of white blood cells and platelets were too low (i.e., similar to background) to be useful as diagnostic measures of human chromium status. Although efforts to reduce background contamination were successful, the very low concentrations of chromium in these cells were difficult to measure accurately. Impact: The static measurement of cellular chromium is inadequate as a routine determination of human

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chromium nutritional status.
Restriction of dietary copper resulted in altered temperature regulatory function of rats acutely exposed to cold air. Copper deficiency was associated with an increased rate of loss of body temperature, decreased enzymatic conversion of the active form of thyroid hormone, triiodothyronine, from thyroxine, and decreased activity of the rate limiting enzyme dopamine beta hydroxylase, a copper-containing protein, needed for increased production of norepinephrine, the key regulator of heat production. A key finding was the identification of decreased transcription and translation of uncoupling protein that is required for heat production and thermogenesis in brown adipose tissue. The lack of induction of uncoupling protein formation is caused by a depressed expression of the genetic message for a specific heat shock protein, HSP 70, in copper deficiency. Importantly, the adverse effects of copper deprivation are ameliorated with copper repletion within three days of copper supplementation. Impact: These findings provide important information explaining the mechanism through which copper regulates energy metabolism, and explain why humans with an inborn inability to absorb copper suffer from hypothermia despite adequate hemoglobin concentration.
Supplementation of men with chromium picolinate while participating in controlled resistance training failed to demonstrate increased strength gain, facilitate loss of body fat and enhance muscle mass accretion. Impact: The results of this controlled study served as the basis for the Federal Trade Commission to rule that claims of propitious effects of chromium picolinate supplementation were without scientific basis. Similarly, the U.S. Pharmacopeia has concluded that chromium picolinate does not promote weight loss, facilitate body fat loss or promote muscle mass gain.
Consumption of dietary magnesium in amounts generally consumed by U.S. women resulted in alterations in energy production during submaximal exercise. Postmenopausal women fed 150 mg of magnesium daily demonstrated an increased oxygen consumption and elevated heart rates during submaximal exercise on a cycle ergometer as compared to a diet providing 350 mg magnesium daily, the recommended dietary intake. Magnesium deficiency was documented with increased losses of magnesium and altered blood ionized magnesium, that is consistent with increased mobilization of magnesium from bone, and decreased skeletal muscle magnesium. Impact: These findings provide the first evidence of diet-induced magnesium deficiency in otherwise healthy adults, and demonstrate that dietary magnesium intake consistent with amounts generally consumed by a majority of U.S. women are inadequate to support moderate intensity physical activities of daily life. Because significant physiological impairments were found at magnesium intakes consistent with national estimates of usual intake, there is a need for increased public health education to bolster dietary magnesium intakes to maintain physiological function and health.
Stearic acid promotes non-heme iron absorption and utilization in iron-deficient animals. Studies in rats and canines repeatedly showed that

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stearic acid enhanced the uptake and transfer of non-heme iron from the intestinal mucosa and increased red blood cell volume and hemoglobin concentration. This beneficial effect occurred at high (30%) and moderate (20%) intakes of stearic acid. Furthermore, the enhancement of iron metabolism with stearic acid did not adversely impact calcium or magnesium status. Impact: Stearic acid may be the uncharacterized component of meat, the "meat factor", that promotes non-heme iron utilization. Although saturated fatty acids generally increase atherogenic risk by increasing serum cholesterol and low density lipoprotein cholesterol concentrations, stearic acid has no adverse effects on cholesterol or lipoproteins. Use of stearic acid in recipes containing non-meat foods may reduce the incidence of iron deficiency anemia in humans.

6. What do you expect to accomplish, year by year, over the next 3 years?

FY 2000: Develop, validate and implement a method for the use of tetrapolar bioelectrical impedance analysis for the assessment of regional muscle mass in humans. A model will be developed in weight-stable adults for determination of upper arm and thigh muscle mass. This model will be tested in adults participating in resistance training to increase muscle mass. This study will determine the advantages of specific impedance instruments (i.e., single or multiple frequency) in the predictive accuracy and sensitivity of muscle and fat determinations. If this approach is successful, a practical method will be available for generalized use in the routine assessment of human nutritional status in national surveys and hospital use with patients gaining or losing muscle and fat mass.
FY 2000: Conclude study of the effects of chromium picolinate supplementation on mineral status and body composition of women consuming controlled diets. This study is examining the hypothesis that chromium picolinate adversely affects iron metabolism; it will also evaluate the independent effect of picolinate per se on mineral metabolism. This project is novel because it will be the first study to examine potential effects of chromium picolinate in subjects with controlled energy intake and expenditure.
FY 2000: Examine the hypothesis that the copper requirement to maintain bone mineral content is increased during weight loss. Previous trials have shown a strong tendency for supplemental copper to attenuate bone mineral loss during weight loss. This study will provide needed data to test the hypothesis that an increased amount of copper is needed to maintain bone mass. Chemical analyses will be completed and statistical analyses of the data will ensue.
FY 2001: Determine the effect of tissue copper depletion independently of anemia on the metabolic perturbation in energy metabolism of copper-deficient rats exposed to exercise training. Refine and implement a new approach to normalize oxygen carrying capacity of copper-deficient rats and examine the effects of copper deficiency on energy metabolism and

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substrate utilization at rest and during controlled exercise in rats.
FY 2001: Determine the effect of type and amount of fatty acids on the transport and uptake of iron and other mineral elements by intestinal cells. Determine the induction and expression of cellular transport proteins of Caco-2 cells in vitro to elucidate the molecular mechanisms of dietary fat on trace element absorption.
FY 2001: Test the hypothesis that restricted copper and iron adversely affect oxidative capacity and oxygen utilization of mitochondria isolated from skeletal muscle and other tissues of rats exposed to specific stressors, including cold and exercise. These studies also will determine if molecular signals, uncoupling proteins and heat shock proteins, are impacted at the level of transcription or expression, by copper and/or iron deprivation.
FY2002: Delineate the effects of dietary zinc and exercise on the induction and expression of zinc-containing enzymes involved in energy production in rodents. By using graded dietary zinc levels and different types of exercise training (endurance and resistance), this study will determine if limiting zinc intake has adverse effects on ability to improve physical performance by limiting the production and activity of specific zinc-containing enzymes.
FY2002: Initiate studies of stearic acid supplementation on iron absorption and utilization of humans with low iron status. These studies will utilize foods high in stearic acid to determine if any benefit can accrue from routine consumption of diets high in stearic acid to promote iron uptake and hemoglobin regeneration in individuals with moderate anemia.

7. What science and/or technologies have been transferred and to whom? When is the science and/or technology likely to become available to the end user (industry farmer, other scientists)? What are the constraints, if known, to the adoption and durability of the technology products

A public presentation on the effects of supplemental chromium on exercise performance and the effects of exercise on chromium requirements was made for the members of the Dietary Reference Intake panel on Trace Elements, Food and Nutrition Board of the National Academy of Sciences. Information on the efficacy and safety of creatine, a popular nutritional supplement used by athletes, was published in the local newspaper, Grand Forks Herald, "Creatine: Consensus or Controversy", April 14, 1999. Information about the basic and applied aspects of the tetrapolar bioelectrical impedance method have been transferred to other federal agencies that are using this technology to derive national body composition norms that will be made available to the public. Results from studies are published in peer-reviewed scientific journals. Upon publication, the methodology, data, and interpretations of the data are immediately available to scientists and other interested parties. In addition, experimental results and educational information pertinent to public interests are made

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National Program(s): 107 100%

available through the local newspaper, The Grand Forks Herald, and statewide through the Interactive Video Network of the North Dakota State University Service Continuing Education Program.

8. List your most important non-peer reviewed publicatons and presentations to non-scientific organizations, and articles written about your work (NOTE: this does not replace your peer reviewed publications which are listed below

A feature story, "Athletes Need Zinc", was published in Agricultural Research, July 1999.
Presentations to non-scientific groups:
U.S. Olympic Training Center, Colorado Springs, CO, January 18, 1999. Lecture: Nutritional Supplements and Physical Performance: Do They Work and For Whom?
University of North Dakota, Department of Health, Physical Education and Recreation, Grand Forks, ND, April 14, 1999. Lecture: Nutritional Supplements and Performance - A Guide for Coaches.
North Dakota Academy of Sciences, Symposium on Nutritional Supplements -- Can Great Performance, Good Health and Long Life Come From A Bottle?, Grand Forks, ND, April 15, 1999. Lecture: Can Nutritional Supplements Enhance Sports Performance?
North Dakota Dietetic Association, Spring Meeting, Grand Forks, ND, April 22, 1999. Lecture: Nutritional Supplements and Physical Performance: Facts and Fantasy.
University of North Dakota Strength and Conditioning Seminar for Coaches, Grand Forks, ND, April 29, 1999. Lecture: Weight Loss and Gain in Athletes - Creatine Supplementation.
North Dakota High School Coaches Association Annual Meeting, Grand Forks, ND, July 27, 1999. Lecture: Ergogenic Aids and Strength Training.
Pan American Games Coaches' Seminar, University of North Dakota Hyslop Center, Grand Forks, ND, July 29, 1999. Lecture: Nutritional Supplements, Training and Performance.
Presentations to Major Scientific or Professional Organizations:
Gatorade International Sports Science Institute Course, Ergogenics: Enhancement of Performance in Exercise and Sport, Caracas, Venezuela, May 15, 1999. Lecture: Vitamins and Minerals and Exercise Performance.
Food and Nutrition Board Forum, Dietary Reference Intakes for Chromium, Copper, Iodine, and Manganese, Washington, DC, April 20, 1999. Lecture: Chromium and Exercise.

9. Scientific Publications:

01. LUKASKI, H.C., MARCHELLO, M.J., HALL, C.B., SCHAFER, D.M. and SIDERS, W.A. 1999. Soft tissue composition of pigs measured with dual x-ray absorptiometry: comparison ... of carcass thickness. Nutrition 15:697-703.

02. LUKASKI, H.C. 1999. Requirements for clinical use of bioelectrical impedance analysis. Ann. N.Y. Acad. Sci. 873:72-76.

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Publications: (Continued)

03. LUKASKI, H.C. 1999. Vitamin and Mineral Metabolism and Exercise Performance. pp. 261-313. IN: Perspectives in Exercise Science and Sports Med., vol. 12. L. Spriet, D.B. Lamb (eds.) Cooper Publ. Group, Carmel, IN.

04. LUKASKI, H.C. 1999. Magnesium, phosphate and calcium supplementation in exercise and sport. pp. 197-210. IN: Microelements, Water and Electrolytes ... Nutr. J. Driskell and I. Wolinsky (eds.) CRC Press. Boca Raton, FL.

05. LUKASKI, H.C. 1999. Chromium. pp. 279-302. IN: Annual Review of Nutrition. D.B. McCormick, D.M. Bier, A.G. Goodridge and R. Parmer (ed.) Annual Reviews Inc, Palo Alto, CA.

06. LUKASKI, H.C., HALL, C.B., MICHELSEN, K.G. and SIDERS, W.A. 1999. Altered metabolic responses during exercise in men fed diets low in zinc. FASEB J. 13: Abstr. p. A214.

07. LUKASKI, H.C. 1999. Can nutritional supplements enhance sports performance? Proc. N.D. Acad. Sci. 53: Short Communication pp. 83-85.

Approved: FORREST H NIELSEN Date: 09/99
Title: CENTER DIRECTOR

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